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CONTENTS

**THE RELATION OF INSPIRATION OF FISHES
TO ENVIRONMENT**

EDWIN B. POWERS, et al.

**CONCERNING CERTAIN PRIMATOLOGICAL
CONCEPTS**

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THE RELATION OF RESPIRATION OF FISHES TO ENVIRONMENT

By

EDWIN B. POWERS *et al*

*From the Department of Zoölogy, University of Tennessee,
Knoxville*

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THE RELATION OF RESPIRATION OF FISHES TO ENVIRONMENT

I. INTRODUCTION

BY EDWIN B. POWERS

In the last analysis, in order that any organism occupy a habitat, it is essential that it be adjusted as to respiration, nutrition, and reproduction. The adjustment as to respiration is continuous, while habitats may be changed for purposes of food gathering or for reproduction. The study of these adjustments, *i.e.*, physiology, in the higher forms at least, has long since passed from the purely qualitative to the quantitative stage, and much exact knowledge has been obtained concerning them. Volumes have been written on the physiology of respiration alone. Most of the work, however, due largely to its application to medicine, has been confined to land animals and in general to mammals.

All animals are essentially aquatic as far as their respiration is concerned. One frequently meets the notion that the respiration of aquatic animals is essentially different from that of terrestrial animals. There are approximately 209 ml of oxygen at 0° C and 760 mm pressure in one liter of air, while the same volume of distilled water under identical conditions contains only 9.7 ml of oxygen. The solubilities of the atmospheric gases, $\text{CO}_2 : \text{O}_2 : \text{N}_2 = 179.7$ (part chemically united) : 4.89 : 2.35, differ markedly. Thus, the relative proportions of these three gases in solution are not the same as in air. For example, one liter of air contains approximately 21.5 times as much oxygen and only 5/9 times as much carbon dioxide as one liter of distilled water. It is no doubt due to these facts that it is sometimes assumed that aquatic animals are hard hit as compared to land animals when carrying on the process of oxygen absorption and carbon dioxide elimination. In this assumption three facts are overlooked. The first and most important is that the atmospheric gases dissolved in a fluid to the point of equilibrium have the same partial tensions as their partial pressures, regardless of the ml of each gas dissolved by the fluid (Henry's Law). Second, all membranes of organs of respiration through which gaseous exchanges take place are continuously bathed with water in aquatic forms and are always covered by a fluid in terrestrial forms. The third fact is that the same laws of solubilities and diffusion of gases are applicable to each, the water being the solvent in aquatic forms and the fluid covering the alveolar epithelium being the solvent in the lung breathing terrestrial forms. Thus in each the gaseous tensions are determined by the gaseous partial pressures of the atmosphere in contact with these fluids. It has long been recognized that the relative densities of a gas in solutions do not determine the tensions of this gas in the solutions but that

the tensions of the gas having a given density in different solutions are determined by the coefficient of solubility of the gas in each of the solvents. That is, an equilibrium of a gas between the two sides of a membrane permeable to the gas will be reached when the densities of the gas on the two sides of the permeable membrane are in the same proportion as the coefficients of solubilities of the gas in the two solvents. To put it still another way, equilibrium is reached when the tensions of the gas are the same in the solvents on the two sides of the membrane regardless of the density of the gas in each of the two solvents. Thus it follows that the physiology of the exchange of gases through the membranes of the respiratory organs of aquatic and terrestrial animals is exactly the same in every detail.

There is an essential difference in the morphology of the respiratory organs of lung and gill breathers. The alveolar space of the lungs is more or less a closed system. The gills of gill breathers are more or less continuously exposed to the water and thus form a more or less open system. Hall (1931) suggests that fishes "have apparently no means by which gas tensions to which their gills are subjected may be altered."

In the lung breathing animals the actual oxygen partial pressure of the alveolar air at any given time is determined by the barometric pressure and the rate and depth of respiration as modified by the rate of oxygen utilization. The carbon dioxide partial pressure of the alveolar air is determined by the rate of carbon dioxide production by the body and the rate and depth of respiration (Campbell, Douglas, and Hobson, 1914) and is not modified by barometric pressure since the carbon dioxide partial pressure of the atmosphere is essentially a vacuum (.035%) at all barometric pressures.¹ The actual carbon dioxide partial pressure is in reality controlled by the alkali in use in the blood at any particular instant which in turn is "fundamentally regulated and determined by the pressure of the oxygen at the altitude of the habitat" (Y. Henderson, 1925). This merely means that the adjustments of the oxygen and carbon dioxide partial pressures of the alveolar air and the blood as a physico-chemical system are interreciprocal. In short, these three, carbon dioxide and oxygen partial pressures of the alveolar air and the blood as a physico-chemical system, reach or tend to reach equilibrium as three opposing systems, *i.e.*, a dynamic equilibrium. We may call this a physiological-dynamic-equilibrium. With gill breathers not being able to control so well the carbon dioxide and oxygen tensions of the water bathing the gills, since it is more of an open system, the burden of adjustment is thrown upon the blood as a physiological-physico-chemical system. This physiological-dynamic-equilibrium merely means that an organism is able, within limits, to maintain an adjustment of its internal environment with its external environment (Powers, 1930, p. 352). In Winton and Bayliss' *Human Physiology*

¹ The only way in which the barometric pressure would affect the carbon dioxide partial pressure of the alveolar air is the effect on the rate of diffusion of the carbon dioxide in the alveolar space.

(1931, p. 139) is found a clear statement of this conception. "The blood, however, plays a far greater part than of an indifferent circulating fluid; and its properties are such that, in itself, it has very important action in stabilizing the composition of the internal environment towards the disturbing influences of activity or of alterations in the external environment." The fluid bathing the alveolar membrane of terrestrial animals or water bathing the gill membrane of aquatic animals constitute the external environment, while the blood, the internal circulating fluid, constitutes the internal environment. In terrestrial animals nothing is known regarding the hydrogen-ion concentration of the fluid bathing the membrane of the alveolar spaces or of its effect, if any, on the organism. It is known, however, that within wide limits the hydrogen-ion concentration of the water bathing the gills of aquatic animals has very little, if any, effect upon the organisms (Powers, 1930 and citations). In lung breathing animals potent factors of the external environment are the oxygen and carbon dioxide partial pressures of the alveolar air, and in gill breathing animals it follows (Powers, 1930, and various papers from 1920 to date) that the potent factors are the oxygen and carbon dioxide tensions of the water bathing the gills. From this discussion it is seen that there is one essential difference in the physiology of respiration of terrestrial and aquatic animals. This difference is that terrestrial animals are able within limits to control the oxygen and carbon dioxide partial pressures of their external environment by the rate and depth of respiration, while aquatic animals have very little—at least far less—control of the oxygen and carbon dioxide tensions of the water bathing their gills. In short, the essential difference between the physiology of respiration of terrestrial and aquatic animals is that terrestrial animals are able to adjust within limits both their external and internal environments while in aquatic animals the greater adjustment must be in the internal environment.

The experiments to be described in the following pages were organized to obtain information from different angles concerning the physiology of respiration of fishes and the behavior of fish blood as a physiological-physico-chemical system.

II. BEHAVIOR OF GASES IN SOLUTION

BY EDWIN B. POWERS

Recently two papers (Guild, 1929, and Gross and Kassel, 1930) discussed interesting data regarding the behavior of gases in solution. Guild reported that "Collodion sacs, 16 mm in diameter and about 60 mm long, filled with water or with aqueous solutions of various salts or gases and firmly closed without inclusions of free gas bubbles, when placed in slowly flowing water direct from the Baltimore city water system in April and May of this year [1929], became completely filled with gas in from six to seven days; then

during the next few days the amount of gas continued to increase until the walls became quite tense from the evident development of a pressure within the sacs markedly greater than atmospheric." He reported further that "The walls of several sacs even gave way." When the gas was analyzed it was found to be made up of atmospheric gases in the same proportion found in the atmosphere. It was also found that these gas-filled collodion sacs were emptied of gas when they were placed in running water that had been previously submitted to a partial vacuum created by a faucet filter-pump until only occasional bubbles were given off. Gross and Kassel pointed out that Guild's observations merely demonstrated the fact that the gases in aqueous solution will pass through a permeable membrane and that in water saturated with air at a pressure greater than atmospheric plus the mechanical pressure of the water (the system being unstable, the gaseous tensions of the solution being greater than the atmospheric pressure plus the hydrostatic pressure of the water itself) gas (air) will be evolved to form bubbles. They also pointed out the fact that "the same processes occur in the reverse direction" when the gas-filled collodion sacs are submerged in flowing, partly degassed water. The tensions of the gases dissolved in the water had been previously brought to less than atmospheric pressure, while the gases within the sacs were always, even when the walls were not tense, subjected to atmospheric plus hydrostatic pressure. In other words, the direction of the diffusion of the gases was merely a demonstration of the behavior of gases in solution.

Henry's Law is "The amount of gas which a liquid will dissolve is directly proportional to the pressure of the gas. This holds for all gases which do not unite with the solvent." Dalton's Law states that "The pressure of a mixture of several gases in a given space is equal to the sum of the pressures which each gas would exert if confined by itself in that space." That is, as it is sometimes stated, "Each gas exerts its partial pressure independently of all other gases present." It seems that it is at this point that confusion arises as to the exact behavior of gases in solution. That is, do gases in solution behave according to their respective partial tensions independently of all other gaseous partial tensions, or do other gaseous partial tensions affect the behavior of a given gas, *i.e.*, in the formation of bubbles? A concise statement regarding this point was not made in either of the discussions cited, although the true behavior of gases in solution is clearly implied by the discussion of Gross and Kassel (1930). In Needham and Lloyd's book (1916, p. 44), *The Life of Inland Waters*, we find the statement, "Birge and Juday report a maximum occurrence of oxygen as observed in the lakes of Wisconsin of 25.5 cc per liter in Knight's Lake on August 26, 1909, at a depth of 4.5 M. This water when brought to the surface (with consequent lowering of pressure by about half an atmosphere) burst into lively effervescence, with the escape of a considerable part of the excess oxygen into the air." Birge and

Juday (1911, pp. 52, 53) in referring to Knight's Lake state, "At a depth of 4.5 M there were 25.5 cc of oxygen per liter of water which was 364.5 per cent saturation. . . . At that depth the hydrostatic pressure is about half an atmosphere and this extra pressure would aid materially in holding the oxygen in solution. It is doubtful whether the amount of oxygen could have risen much higher without some of it being liberated in bubbles. . . ." Here again the implication is clear. However, the questions arise: Under just what conditions do bubbles appear beneath the surface of water and what is their gaseous composition? Was the production of bubbles brought about by the partial tension of the oxygen in solution which was 3.645 times its partial pressure in the atmosphere or was it the total or sum of the partial tensions of all the gases in solution that brought about the production of bubbles?

It was because of this seeming confusion in some instances of the behavior of gases in solution and because of want of a logical explanation as to how natural waters (rivers) with a relatively high or low oxygen content and a relatively low or high carbon dioxide tension could maintain this relation after miles of flow (Powers and Hickman, 1928; Powers, 1928) that the author performed a few simple experiments designed to demonstrate the behavior of gases in solution. A clear understanding of the behavior of gases in solution is also of prime importance in an explanation of the deposition of gases into the swim-bladder of fishes and in an intelligent discussion of the physiology of respiration of fishes.

It suffices here to point out the conditions under which bubbles will appear under the surface of the water. Distilled water was aerated with laboratory atmospheric air for one and a half hours at 19.5° C and 725.2 mm Hg. Six 150 ml glass stoppered bottles were filled with this aerated water and aerated for two hours with gas of various nitrogen and oxygen ratios ranging from pure nitrogen to pure oxygen. The six bottles were exposed unstoppered to an atmospheric pressure of 650.2 mm Hg and observed for the appearance of bubbles. Table I gives the number of minutes after exposure to the lower pressure before bubbles appeared. In these and all subsequent observations the first bubbles appeared near the tops of the bottles. Oxygen contents only were determined at the end of the experiment. It is presumed that the total of the gaseous partial tensions of the solutions were the same as the total partial pressures of the atmosphere above the surfaces of the solutions at the end of aeration, but the relative tensions of the gases in solution were obviously not the same as the gaseous partial pressures of the atmosphere, as is shown by the oxygen contents of the different solutions at the end of the experiment.

Five 150 ml bottles of distilled water were aerated for four hours at 23° C and 726.4 mm Hg pressure with gases of various nitrogen and oxygen ratios ranging from pure nitrogen to pure oxygen. The bottles were allowed

to stand unstoppered in the laboratory for over twenty-four hours. During this time the temperature had fallen to 21° C and the atmospheric pressure had risen to 728.5 mm Hg. No bubbles appeared. The bottles unstoppered were then exposed to a pressure of 699.8 mm Hg at 20° C. Bubbles appeared as indicated in column three, Table II.

Finally, distilled water was aerated for four and a half hours with laboratory air at 18° C and 731.5 mm Hg to bring it into equilibrium with the air. The aerated water was placed in six 150 ml glass stoppered bottles and aerated with gases of various nitrogen and oxygen ratios from pure nitrogen to pure oxygen for two hours. During that time the pressure had fallen to 730.3 mm Hg and the temperature had risen to 20.5° C. The bottles of water were then raised to 29.5° C in twelve minutes and allowed to stand in the heated water bath. Column three of Table III shows the number of minutes before bubbles appeared. Column two shows the oxygen contents at the close of the experiments.

From these experiments (Tables I and II), it is seen that bubbles appear in an aqueous gaseous solution when subjected to a reduced mechanical pressure, that is, hydrostatic plus atmospheric pressure. The appearance of these bubbles is independent of partial tensions of individual gases in solution but is dependent upon the total partial tensions of these gases. When their total tensions are equal to or less than the mechanical pressure no bubbles appear, even after standing for more than twenty-four hours. Bubbles will appear when the internal total gaseous tensions are raised above the mechanical pressure; that is, by raising the temperature of the solution.

Bubbles formed below the surface are not composed of one gas but of all gases dissolved and are in proportion to their respective tensions as modified by their respective rates of diffusion, etc. This point is plainly demonstrated by the chemical analysis made by Patrick (Guild, 1929) of the gases contained in the collodion sacs.

Natural waters will tend to maintain their gaseous partial tensions complex even with a rise in temperature except as diffusion takes place at the water-atmospheric surface. At all inclosed surfaces, such as surfaces formed when the total internal gaseous tensions are greater than the mechanical pressure, the complex of the gases lost is determined by the gaseous solution complex partial tensions and their respective rates of diffusion. Even in the inclusion of bubbles of atmospheric air the complex of the gases lost or gained is modified by the gaseous solution complex and if the bubbles grow in size the effect of the gaseous solution complex soon dominates. This is true to a small extent at the water-atmospheric surface. At the same time the temperature is rising due to the water reaching lower altitudes and warmer surroundings, the atmospheric pressure (*i.e.*, the mechanical pressure) is also rising with the lowering of altitude. This increased mechanical pressure

tends to hold the gases in solution at the higher tensions brought about by the rise in temperature. Thus instead of the gaseous partial tensions assuming the partial pressures of the atmosphere, the differences would be intensified. The carbon dioxide especially, due to the fact that natural waters are generally buffered on the alkaline side, resists a change in tension more tenaciously since it enters into chemical combination with the water and alkalies and alkaline earths, thus forming a reserve base of supply. For the same reason its tension would increase more rapidly with rise of temperature than that of other gases. A stream laden with carbon dioxide would thus, under normal conditions, tend to increase its carbon dioxide tension along its course rather than lower it despite the fact that carbon dioxide may continuously be given off. On the other hand, if the stream for any reason should lower its temperature along its course, its carbon dioxide tension would, for the same reason, actually decrease more rapidly than that of any of the other gases in solution. The carbon dioxide tensions were found to be lower than the carbon dioxide partial pressures in the atmosphere by Powers and Hickman *(1928) in Clark Fork of Columbia River, Superior, Mont., and by Powers (1928) in Boulder River, Big Timber, Mont., Jefferson River, Jefferson Island, Mont., St. Regis River, St. Regis, Mont., Pitt River, Baird, Calif., and McCloud River at its mouth, Calif. The effect of a sudden change in the alkali reserve on the carbon dioxide tension of the water by the junction of streams is not known. For further discussion see Powers, 1930, and citations.

From the foregoing discussion it is apparent that the original source of a stream affects its gaseous complex throughout its whole course. Fishes in different streams, as far as the physiology of their respiration is concerned, are living in environments of entirely different atmospheric compositions.

TABLE I

Data show the time necessary for bubbles to appear in different samples of water exposed to reduced pressure. Column two gives the oxygen contents of the different samples and are indices of the gas mixtures used to equilibrate the water at the higher pressure.

Aerated at 19.5° C. and 725.2 mm Hg. Barometric pressure reduced to 650.2 mm Hg at 18.5° C.

<i>Gas used in aeration</i>	<i>Oxygen in ml per liter</i>	<i>Minutes before bubbles appeared</i>
Nitrogen	0.86	13
Oxygen Mixture, Number 1.....	2.85	12
Oxygen Mixture, Number 2.....	6.60	15
Oxygen Mixture, Number 3.....	9.93	15
Oxygen Mixture, Number 4.....	14.26	14
Pure Oxygen	15.72	13

TABLE II

Data show the same things as Table I.
Aerated at 23° C. and 726.4 mm of Hg. Exhausted to 699.8 mm of Hg at 20° C.

<i>Gas used in aeration</i>	<i>Oxygen in ml per liter</i>	<i>Minutes before bubbles appeared</i>
Nitrogen	0.88	30
Oxygen Mixture, Number 1.....	4.24	30
Oxygen Mixture, Number 2.....	8.54	31
Oxygen Mixture, Number 3.....	12.88	33
Pure Oxygen	16.06	25

TABLE III

Data show the same things as Table I except the temperature of the samples of water was raised instead of reducing the pressure.

The temperature was raised from 20.5° C. at 730.3 mm Hg to 29.5° C. at 730.3 mm Hg in twelve minutes.

<i>Gas used in aeration</i>	<i>Oxygen in ml per liter</i>	<i>Minutes before bubbles appeared</i>
Nitrogen	0.39	24
Oxygen Mixture, Number 1.....	2.07	26
Oxygen Mixture, Number 2.....	3.75	20
Oxygen Mixture, Number 3.....	6.59	23
Oxygen Mixture, Number 4.....	11.25	26
Pure Oxygen	20.14	22

III. METHODS USED IN DETERMINING THE OXYGEN CONTENT AND THE CARBON DIOXIDE TENSION OF EXPERIMENTAL WATER

BY EDWIN B. POWERS

The oxygen content of the experimental water bathing the fish was determined by the Winkler method as modified by Birge and Juday (1911). But instead of titrating the whole content of a collecting bottle for the iodine liberated, 50 or 100 ml were measured from the collecting bottle and titrated. When nearly uniform collecting bottles were used, the error due to adding constant volumes of reagents to the slightly different volumes of water was less than the experimental error of the Winkler method itself.

The oxygen in solution is not expressed in tensions but in ml per liter. The relative oxygen contents of the water were taken to be the same as the relative oxygen tensions of the water. The error, due to slight variations in relative oxygen contents because of the variations in the coefficient of solubility of oxygen brought about by variations in temperature and the slight variations in the complex of the substances in solution of the different experimental waters, is not very great and was not taken into consideration.

Although the oxygen in ml per liter is taken to mean the relative tension of the oxygen, we are well aware that in the physiology of respiration of fishes it is the actual tension of the oxygen of the external environment that is of importance and not the actual oxygen content of the water. This also applies

to carbon dioxide. It is the tension of the gas that determines the diffusion of the gas through a permeable membrane and not the actual amount of the gas in solution. Some biologists have measured and continue to measure carbon dioxide contents of natural waters and consider this as measurement of an environmental factor, despite the fact that physiologists of lung breathing animals measure the carbon dioxide partial pressures of alveolar air and obviously consider the actual carbon dioxide contents of the fluid bathing the alveolar membrane in ml per liter as insignificant, as they do not even mention the possibility of this being a factor in the physiology of respiration. Keys (1930a) in his paper on methods for determining oxygen consumption of fishes, does not measure carbon dioxide tension and does not mention the desirability of doing so. The pH of the water and the ml of carbon dioxide of the water have been confused with carbon dioxide tension. The pH as such is not a measure of the carbon dioxide tension of the water (Powers 1927, 1928a, 1930; Powers and Bond 1927, 1928).

The carbon dioxide tension of the water was determined by the colorimetric method described by Powers (1927, 1930) and Powers and Bond (1927, 1928). The formula $pH = -ne - n\log P$ (Powers 1930) was used to calculate the carbon dioxide tension of the water measured in percentage of an atmosphere under standard conditions. n and e are constants, both of which vary with different waters. Theoretically ne is equal to the pH of the water with a carbon dioxide tension of one atmosphere. Both n and ne increase in value with an increase in alkali reserve of the water.

Since there are two unknown quantities, n and e , pH readings must be taken at two known carbon dioxide tensions in order to calculate the unknown carbon dioxide tension of the water from the pH reading of the water (Powers, 1927, 1930; Powers and Bond, 1927, 1928). The pH of the water with the unknown carbon dioxide tension was first read. A sample of the water was then aerated with air containing .03% carbon dioxide and pH read. Another sample of the same water was aerated with air containing 9.6% carbon dioxide and the pH again read. From these data the unknown carbon dioxide was calculated. To use the formula $pH = -ne - n\log P$ the carbon dioxide tension must be expressed in atmospheres. In the following pages we have expressed the carbon dioxide tensions in mm of Hg instead of in atmospheres.

IV. RELATION OF CARBON DIOXIDE AND OXYGEN CONTENTS OF THE BLOOD TO THE CARBON DIOXIDE AND OXYGEN TENSIONS OF THE ENVIRONMENTAL WATER

BY EDWIN B. POWERS, FLORIAN G. HOPKINS, AND
THRESSA A. HICKMAN

Experiments were planned to determine if possible the effect of the oxygen content (tension) and the carbon dioxide tension of the water bathing a fish upon the oxygen and carbon dioxide contents of its blood. Very definite relations have been found between the oxygen and carbon dioxide partial pressures of the external environment—the alveolar air—and the oxygen and carbon dioxide contents (tensions) of the blood of lung-breathing animals. Very little is known regarding these relationships among aquatic, gill-breathing animals.

MATERIALS AND METHODS

Observations were made on the blue cat, *Ictalurus punctatus* (Rafinesque) and the German carp, *Cyprinus carpio* Linnaeus. The carp were taken from a small artificial lake and kept in a small artificial pool supplied with running, well aerated city supply water and were used as needed. The blue cat were taken from a trap in the Holston River and were generally placed in the experimental water soon after being brought to the laboratory or were placed temporarily in the artificial pond. All specimens used weighed from three fourths of a pound to two or two and one half pounds.

The experimental fish was placed in a five gallon museum jar filled with tap water. The water was continuously aerated with an air mixture of known nitrogen, oxygen, and carbon dioxide content during the progress of the experiment, which was terminated after two and a half to about seven hours. The fish was held below the surface of the water by a screen to prevent it swallowing air. Care was also taken to prevent the fish from swallowing air bubbles introduced for aeration.

At the end of an experiment the temperature was taken and the oxygen content and the carbon dioxide tension of the water was determined as has already been described (p. 394). Blood was collected directly from the heart by means of a 0.2 ml Ostwald-Van Slyke pipette with a Luer syringe attached to one end and a sharp pointed needle to the other. Potassium oxalate was used as the anticoagulant. The 0.2 ml of the blood was immediately transferred to the Van Slyke constant volume blood gas apparatus (Van Slyke and Neill, 1924; Harington and Van Slyke, 1924; and Van Slyke, 1927). A second sample was generally obtained by a second 0.2 ml Ostwald-Van Slyke pipette, Luer syringe and needle and immediately transferred to a second Van Slyke constant volume blood gas apparatus. The carbon dioxide and oxygen contents of the two samples were then determined simultaneously by two of

us. A second sample or even a first sample was not always obtained because of the struggling fish, the weak circulation, and the small amount of blood actually present.

During the heart punctures the fish were kept beneath the surface of the experimental water and the gills were never allowed to come in contact with the air. The blood samples were, thus, more nearly true representations of the blood under the conditions of the experiment. The term "blood" in this paper denotes venous blood, as no other blood was used in these experiments.

EXPERIMENTAL DATA

The experimental data have been arranged in four different ways. The data in Tables IV and VIII are arranged according to the oxygen increase in the experimental water, Tables V and IX according to the carbon dioxide tension increase of the water, Tables VI and X according to the oxygen increase content of the blood, and Tables VII and XI according to the carbon dioxide increase content of the blood.

The four way arrangement of data is chosen because it is simple, and each of the variables can be compared with each of the other three.

DISCUSSION OF EXPERIMENTAL DATA

When the data are arranged according to an increase in the oxygen content of the water, Tables IV and Figure 1, an increase in the volume per cent of the carbon dioxide content of the blood of the blue cat with increase in the oxygen content of the water is observed. In Table VII and Figure 4, where data are arranged according to the increase in volume per cent of carbon dioxide in the blood, the higher oxygen contents of the water are found when the volume percentages of the carbon dioxide of the blood are higher. In Table VIII and Figure 5 giving data on the carp blood, the same tendency is shown, except in Experiments 60, 44, and 51. In Table XI and Figure 8 the tendency for the higher oxygen content experiments to coincide with the experiments in which the volume per cent of carbon dioxide is high is still less obvious. The exceptions are Experiments 55, 56, 60, and 44. The last two appear as exceptions in both tables and figures. All carbon dioxide tensions of the experimental water of the carp were 2.70 mm Hg and above, which are more than ten times as great as the carbon dioxide partial pressure of the atmosphere. We have reasons to believe that the red blood corpuscles of the carp will not stand up so well under adverse conditions as those of the blue cat. Others (Kokubo, 1927) have found difficulty in handling carp blood. This will be further discussed later.

When the data are arranged in the order of increase in the carbon dioxide tension of the water (Table V, Figure 2), it is seen that the volume per cent of the carbon dioxide of the blood of the blue cat increases with an increase

in the carbon dioxide tension of the water. This increase is not as marked as was the increase in the volume per cent of the carbon dioxide of the blood with increase in the oxygen content of the water. Again Table VII and Figure 4 show that the volume per cent of the carbon dioxide of the blood increases with the increase in the carbon dioxide tension of the water. The exceptions are Experiments 10, 38, 15, 37, 2, 35, 11, 38, and 19, all of which are low oxygen content water experiments, the significance of which will be discussed presently.

Table IX and Figure 6, which present data for carp blood, show a more marked increase in the volume per cent of carbon dioxide in the blood with increase in carbon dioxide tension of the water than did the blue cat. Table XI and Figure 8 show an increase in the carbon dioxide tension of the water with an increase in the carbon dioxide volume per cent of the blood. This again is more marked than in the blue cat. Table IV and Figure 1 show an increase in the volume per cent of oxygen of the blood with an increase in the oxygen content of the water. Table VI and Figure 3 show that the higher oxygen contents of the water tend to coincide with an increase in the oxygen volume per cent of the blood. Tables VIII and X and Figures 5 and 7 show that there is a very slight relation between the oxygen content of the water and the oxygen volume per cent of the blood of the carp.

Tables V, VI, IX, and X and Figures 2, 3, 6, and 7 show very little if any relation between the oxygen volume per cent of the blood and the carbon dioxide tension of the water for either the blue cat or carp. On the other hand the oxygen volume per cent of the blood of both these fishes is controlled more by the oxygen content of the water as indicated by the close paralleling of the volume per cent of oxygen in blood lines with the oxygen ml per liter lines in Figures 2 and 6, there being only a few marked exceptions.

SUMMARY OF EXPERIMENTAL DATA

The volume per cent of the carbon dioxide of the blood of the blue cat increases with an increase in the oxygen content of the water. This is less obvious in the case of the carp.

There is an increase in the volume per cent of the carbon dioxide of the blood of both the blue cat and the carp with an increase in the carbon dioxide tension of the water. This tendency is more marked in the carp than in the blue cat.

The volume per cent of the oxygen in the blood of the blue cat increases with the oxygen content of the water. This relation if present in the carp is very slight.

There is a very slight, if any, relation between the volume per cent of the oxygen in the blood and the carbon dioxide tension of the water in either the blue cat or carp.

The volume per cent of the oxygen of the blood of both the blue cat and carp is controlled more by the oxygen content than by the carbon dioxide tension of the water.

There is a paralleling of the volume per cent of the carbon dioxide and the volume per cent of the oxygen of the blood of both the blue cat and the carp. However, it is less obvious in the carp. That is, the two seem to have a reciprocal relation up to between 6 and 6.5 ml oxygen per liter after which the two run parallel with one exception, Experimental 54. In the blue cat the reciprocal relation between the oxygen and carbon dioxide volume per cents of the blood at very low oxygen contents of the water is not noted up to about 1 ml per liter. The parallelism is quite marked at all higher oxygen contents of the water. See Table IV and Figure 1 for data of the blue cat and Table VIII and Figure 5 for data of the carp. When the oxygen per liter in the water, the volume per cent of carbon dioxide in the blood and the carbon dioxide tension of the water are plotted against the volume per cent of oxygen in the blood of the blue cat, it is seen that the volume per cent of carbon dioxide in the blood is controlled more by the carbon dioxide tension of the water than by the oxygen content. See Table VI and Figure 3. When the volume per cents of oxygen and carbon dioxide of the blood of the blue cat and the carbon dioxide tension of the water are plotted against the carbon dioxide tension of the water, Figure 2, data taken from Table V, there is shown a marked parallelism in the three—volume per cents of carbon dioxide and oxygen of the blood (the blue cat) and the oxygen content of the water.

The interrelations of the volume per cents of the oxygen and carbon dioxide of the blood, the oxygen content of the water and the carbon dioxide tension of the water can best be summarized in the case of the blue cat by referring to Tables IV and V and Figures 1 and 2. There is a marked parallelism of the other three when plotted against the oxygen content of the water and when plotted against the carbon dioxide tension of the water. Table VI and Figure 3 show that the volume per cent of carbon dioxide of the blood is controlled more by the carbon dioxide tension of the water than by the oxygen content. There are some marked exceptions and those are at very low oxygen volume per cent of the blood. Table VII and Figure 4 show a more marked parallelism in the volume per cent of oxygen in the blood and the oxygen content of the water above about 1 ml per liter than with the carbon dioxide tension of the water. The same interrelations are shown in the carp. See Tables IX and X and Figures 6 and 7.

In short, the findings of these experiments are: first, the volume per cents of oxygen and carbon dioxide of the blood are increased or decreased with an increase or decrease in the oxygen content of the water; second, the carbon dioxide volume per cent of the blood is increased or decreased with an increase or decrease in the carbon dioxide tension of the water; and third, if the

volume per cent of oxygen of the blood is modified by an increase or decrease in the carbon dioxide tension of the water it is not at all obvious in these experiments. It must be remembered again that in these experiments we are dealing entirely with venous blood and not arterial.

DISCUSSION

The findings of these experiments are a complete substantiation of the conclusion of Powers and Logan (1925) that "The alkaline reserve of the blood plasma of the blood . . . is increased by a high CO_2 tension and decreased by a low CO_2 tension of the medium seemingly independent of the pH as such" which has been criticised by Jobes and Jewell (1927). This criticism by Jobes and Jewell has been discussed elsewhere by one of us (Powers, 1930) and would not be mentioned again here except for the fact that Creaser (1930) has accepted Jobes and Jewell's criticism as valid. We will state, however, that Creaser's paper was published before the publication of the paper by Powers (1930), in which it was explained that "the data in Table I (Powers and Logan, 1925) are arranged in decreasing carbon dioxide partial pressures of the air used for hours to aerate the water in which the fish were placed. Air of different carbon dioxide partial pressures was resorted to, since the carbon dioxide tension of the water could not be measured rapidly. The data cannot be rearranged as in Table II (Jobes and Jewell, 1927) according to the titratable alkalinity (Johnston, 1916) and indicate comparative carbon dioxide tensions accurately by the pH of the water." The independence of carbon dioxide tension of natural waters to their pH's is shown in Table I and Figure 1 of Powers' paper (1930).

Our experiments here presented are ill adapted to test the effect of the pH of the water as such upon the alkali reserve, *i.e.*, the carbon dioxide capacity, of either the blood plasma or the whole blood, since tap water, the same kind of water, was used in all the experiments. In the same kind of water the pH and carbon dioxide tension have a definite relation to each other (Powers, 1927, 1928, 1930, and Powers and Bond, 1927, 1928) and it could not be known which of the two was the controlling factor. However, it is the general consensus of opinion of workers and observers that the pH as such over wider range has very little or no effect upon aquatic animals.

The substantiation of the conclusion, "a low oxygen tension of the medium causes an increase in the alkaline reserve of the blood plasma of the fish, due perhaps to the reduced hemoglobin being less acid than oxyhemoglobin. This tendency is much more marked in the high CO_2 tensions" (Powers and Logan, 1925) is not quite so obvious. However, the carbon dioxide volume per cent of the blood is higher in proportion to the carbon dioxide tension and the oxygen contents of the water up to an oxygen content of the water of about 1 ml per liter. There is one exception. In Experiment 38 in which

there is a high carbon dioxide tension of the water, there is a comparatively low carbon dioxide volume per cent of the blood. The fish in this experiment had to be removed at the end of two and one half hours, since it showed signs of asphyxiation. It seemed that it was only a matter of minutes until the fish would have been dead. Perhaps its compensating mechanism had completely broken down. It has long been known that fishes suffer marked oxygen want at low oxygen tension of the water.

"Laboratory experimentation shows that oxygen want is felt neither by fresh water nor marine fishes until the oxygen content of the water has been reduced to about 1.7 to 0.4 cc oxygen per liter (Duncan and Hoppe-Seyler, 1893, Chlopin and Nikitin as cited by Kupzis in 1901, König and Hünnemeier 1901, Kupzis 1901, Winterstein 1908, Henze 1910, Gardner and Leetham 1914, and 1914a, and Gaarder 1918)" (Powers, 1921)—a statement obviously overlooked by Keys (1930). In the work (Powers and Shipe, 1928) cited by Keys, it seems the point was missed that Powers and Shipe were attempting to determine the effect on the rate of oxygen consumption of fishes immediately after being suddenly exposed to waters of various combinations of carbon dioxide and oxygen tensions and that they were not attempting to determine the normal rate of oxygen consumption of fishes in water of various combinations of carbon dioxide and oxygen tensions after the fishes had become acclimatized. Campbell (1925) has shown that the oxygen consumption of rabbits is lowered when suddenly exposed to low oxygen partial pressures. He further states (1927a), "In previous experiments with unacclimatized, immobilized animals a sudden decrease of O₂-pressure in the air to below 84 mm Hg (12 p.c.) for a few hours definitely lowered O₂-consumption. This was apparently a temporary effect, since in the prolonged exposure to gradually lowered O₂-pressure no definite effect upon O₂-intake was observed. . . ." The oxygen consumption of an unacclimatized rabbit exposed to an O₂-pressure of about 66 mm Hg. (9.4 p.c.) had been lowered by about 25 p.c. by the seventh day. The rabbit died on the ninth day of exposure. Another partly acclimatized rabbit withstood 10 days with one day's interval of breathing ordinary air. This rabbit, after exhibiting signs of tetanus on the tenth day recovered completely "in time," after being brought back to normal oxygen pressures.

A knowledge of the fact whether or not fishes suffer from oxygen want when suddenly exposed to a different complex of carbon dioxide and oxygen tensions is very important in attempting an explanation of the migratory paths of fishes, such as the herrings and salmons, and working out means by which these fishes can be perpetuated.

All observations have been made on venous blood and not arterial. The volume per cents of oxygen and carbon dioxide do not represent the blood content after passing through the capillaries of the gills and being equi-

librated with the gaseous contents of the water but represent the average of what is left in the blood after passing over the tissues of the body. These reflect but do not measure the oxygen and carbon dioxide tensions of the arterial blood of the fishes after having been exposed to waters of various complexes of oxygen and carbon dioxide tensions. Campbell (1927) showed that prolonged exposures of certain mammals to low oxygen pressures depressed the carbon dioxide and oxygen tensions in the tissues. The reverse was found to be true when the oxygen pressure of the air breathed was increased. The fact that the volume per cent of carbon dioxide is increased with an increase in the oxygen tension of the water is in direct keeping with the findings of the physiologists of terrestrial mammalian animals summarized by Y. Henderson (1925). "The amount of alkali in use in the blood of a healthy individual is profoundly regulated and determined by the pressure of oxygen in his lungs at the altitude at which he lives. In other words, the type of blood that he has in his vessels at any one time is a function of the mean barometer at the place where he is then living." Campbell (1927) showed through experimentation that blood alterations were due to oxygen pressure independent of barometric pressure. This oxygen tension of the water effect should then be independent of the hydrostatic pressure upon the fish. The gas content and its coefficient of solubility, and not hydrostatic pressure, determines the tension of the gas in solution.

It is now common knowledge (Douglas, Haldane, Henderson, and Schneider, 1913, and substantiated by various workers) that the red corpuscles increase with lowering the oxygen pressure below that of air at sea level and decrease again to sea level normal with a rise of oxygen pressure back to that at sea level. Campbell (1927) proved that the red corpuscle response was independent of barometric pressure and that a further increase in oxygen pressure above that at sea level will call forth a further decrease in the number of red corpuscles. Kawamoto (1929 and 1929a), Hall (1928), and Hall and Gray (1929) have called attention to the variations in the number of red corpuscles in various species of fishes and in the same species of fishes under different conditions. Hall (1928) showed that the number of red corpuscles increased with length of time of asphyxiation and Kawamoto (1929) showed that there was a seasonal variation in the eel.

Since about one half (Van Slyke, 1921) of the carbon dioxide of the blood is carried by the red corpuscles, and since the number of red blood corpuscles are actually decreased (see p. 414) with an increase in the oxygen tension of the water, the calling forth of an increased alkali into the blood with increase in oxygen tension of the water is even more striking than indicated by the figures.

In addition to the effect of oxygen tension of the water upon the volume per cent of carbon dioxide of the blood there is a direct effect of the carbon dioxide

tension of the water. The volume per cent of the carbon dioxide of the blood runs parallel with the carbon dioxide tension of the water, except as modified by the oxygen tension of the water. This direct carbon dioxide effect, independent of the oxygen pressure upon the alkali of the blood, has not been demonstrated in mammals as far as we are aware. However, Hooker, Wilson, and Connett (1917), Scott (1918), and Gesell (1925 and various papers) have shown a direct carbon dioxide effect upon the respiratory center, and all workers on the physiology of respiration agree that the immediate control of the ventilation is by the carbon dioxide tension of the alveolar air (Haldane, 1922, and citations). In mammals the carbon dioxide partial pressure of the lungs is lowered in acclimatization (P. Bert, 1878, Mosso, 1898, Douglas, Haldane, Henderson, and Schneider, 1913, Haldane, 1922, Barcroft, Binger, Bock, Doggart, Forbes, Meakins, and Redfield, 1923, Barcroft 1925, Schneider, 1921, Campbell, 1927, and almost all, if not all, who have worked on acclimatization) to lowered oxygen pressure. There have been some differences of opinion as to the cause of the lowered carbon dioxide partial pressure of the alveolar air. FitzGerald (1913, 1915) determined that the alveolar CO_2 partial pressure varies in the same direction and nearly with the barometric pressure of the air, that is, with the oxygen partial pressure of the air breathed (Y. Henderson, 1920). Y. Henderson (1920, 1925) pointed out that in the same individual when completely or almost completely adjusted to altitudes from sea level to 14,000 ft. there was a very close proportionality between the oxygen and carbon dioxide pressures in the lungs. He further states, "If this were not the case the pH would have had widely varying values in acclimatization to various altitudes." Barcroft (1925) calls attention to the very obvious fact that the individual is exposed to an almost vacuum, $0.035 \pm \%$, of carbon dioxide regardless of the altitude that he is inhabiting. In the habitats of fishes the carbon dioxide tension of the water does not vary with the altitude, up or down, nor does it maintain a nearly definite relation to the oxygen tension of the water (Powers and Hickman, 1928, and Powers, 1928). It is shown by the data presented in this paper that the carbon dioxide capacity of the blood as indicated by the carbon dioxide volume per cent of venous blood, is also modified by the carbon dioxide tension of the blood. It is obvious that if this were not controlled by the oxygen tension of the water and did not respond to the carbon dioxide tension of the water, the pH of the blood of fishes would have different values in waters with different combinations of carbon dioxide and oxygen tensions. As far as we are aware, no one has yet determined the stability of the pH of fish blood. One would conclude, however, from work done on other bloods that the pH of fish blood would be fairly constant.

The fact that the amount of alkali is affected by both carbon dioxide and oxygen tension of the water in which the fish finds itself makes the mechan-

ism for maintaining a physiological-physico-chemical equilibrium among the alkali in use in the blood, the tensions of oxygen and carbon dioxide in arterial and venous blood and the number of red blood corpuscles more complex than in terrestrial lung breathing animals. Campbell (1927) shows that in mammals the tissue oxygen tension is lowered with decreased oxygen pressure. It is generally accepted as a fact, a discussion of which can be found in any advanced textbook of physiology (Bayliss, 1924, p. 622), that loading of the hemoglobin and the unloading of the oxyhemoglobin is affected by the hydrogen ion concentration of the blood plasma. It is more reasonable to suppose that in reality it is the hydrogen ion concentration of the stroma of the red blood corpuscles that determines the per cent of saturation of oxyhemoglobin and not the hydrogen ion concentration of the blood plasma as such (Barcroft, 1925), and that the point of saturation, *i.e.*, the per cent of saturation, of the hemoglobin-oxyhemoglobin at a given hydrogen ion concentration, is determined by the oxygen pressure. In short, if the oxygen pressure is not such as to place the oxygen per cent concentration of the oxyhemoglobin-hemoglobin at a value most efficient in liberating oxygen with a given rise in hydrogen ion concentration, the amount of oxygen is not sufficient to supply the oxygen demand of the fish. The fish will suffer from oxygen want. In normal animals, the deficiency is made up in two ways. First, the rate of flow of the blood can be increased or decreased, and second, the number of red blood corpuscles can be increased or decreased. A third possible means is to utilize oxygen at lower pressure in the tissues. This of itself would bring more alkali into play, since hemoglobin is a weaker acid than oxyhemoglobin (Christiansen, Douglas and Haldane, 1914). In the physiological-physico-chemical equilibrium there is another complicating factor. The alkali reserve of the water determines the change in carbon dioxide tension with the absorption of a given amount of carbon dioxide per volume of water. That is, the alkali reserve of the water in which a fish is found determines the efficiency of the elimination of carbon dioxide from the blood when the gills are bathed with water at a given rate. Although the fishes might be able to control within limits the oxygen and carbon dioxide tensions of the water bathing the gills by the rate of respiration, the matter of regulation is much more complex than in mammals, due to the fact that the carbon dioxide and oxygen tensions of the water do not always vary in the same direction and that a given amount of carbon dioxide does not bring about the same amount of change in the carbon dioxide tension of a given volume of water when added to it. This pH (Shelford, 1929) effect, *i.e.*, the pH of the water equilibrated with air (Powers, 1930), has never been investigated. Thus, whether there is or is not some kind of an effect of the pH upon fishes remains an open question.

TABLE IV

Data showing the interrelations between the oxygen content and carbon dioxide tension of the water and the oxygen and the carbon dioxide volume per cents of the blood of the blue cat, *Ictalurus punctatus*, (Rafinesque), when arranged according to an increase in the oxygen content of the water.

Expt. No.	O ₂ in ml per 1 in water	Vol. % O ₂ in blood	Vol. % CO ₂ in blood	CO ₂ in mm Hg in water	Time in hours	Temperature °C.
16	.35	.09	6.64	2.31	4.50	27
19	0.63	0.34	11.52	2.84	5.00	29
38	0.67	0.42	6.06	11.08	2.50	34
15	0.90	0.40	7.63	2.94	6.00	30
9	0.93	1.18	15.77	4.15	7.00	29
14	0.96	0.36	13.10	3.02	5.33	29
36	0.97	0.73	0.55	9.66	3.70	32
37	1.00	0.82	9.32	4.92	4.30	32
17	1.02	0.09	6.44	2.66	5.00	27
18	1.21	0.61	4.54	2.47	3.00	29
10	1.24	1.35	1.35	17.25	2.61	28
2	1.35	1.36	1.35	9.33	2.08	30
35	1.39	0.85	9.37	4.53	3.50	31
3	1.58	1.03	12.49	2.21	4.25	30
11	1.91	1.45	9.59	2.90	6.50	29
13	1.97	1.30	13.22	2.93	7.25	28
6	2.01	4.15	17.04	3.15	4.50	29
20	2.12	0.25	9.75	2.88	3.80	32
8	2.20	0.76	13.34	3.74	5.50	29
12	2.35	1.32	14.59	2.93	5.75	28
7	2.65	0.86	13.73	2.30	4.80	31
4	2.70	1.00	12.43	2.06	4.25	30
26	3.00	2.41	22.13	5.11	4.50	33
39	3.10	3.97	13.19	1.63	5.00	33
1	3.32	0.36	10.95	1.22	2.60	33
5	3.43	0.93	12.46	3.50	3.50	27
40	3.60	4.35	12.17	1.92	3.60	33
42	4.76	0.49	11.85	4.18	3.08	27
23	5.97	3.92	3.77	20.65	8.57	29
24	5.97	3.62	3.62	22.00	4.80	29
21	10.20	4.00	10.31	17.87	2.19	3.80
22	10.42	3.73	3.86	16.42	2.19	4.30
30	10.82	3.64	19.48	3.71	4.25	20
33	13.27	5.39	18.38	3.26	4.00	28
34	14.42	5.42	18.82	3.59	5.00	28
29	14.58	2.93	17.16	3.62	3.75	27
28	15.46	2.90	18.68	3.78	2.70	26
27	15.83	4.15	17.04	3.15	3.50	25
32	19.96	5.78	18.48	3.81	4.50	28
31	22.65	5.79	18.49	3.85	3.75	28

16 and 17. Fish almost dead. Labored breathing.
 23. Fish almost dead. Labored breathing.
 30 and 31. Fishes struggled violently.
 33. Fish struggled.
 38. Fish almost dead at end of experiment.
 42. Fish leaped from experimental water.

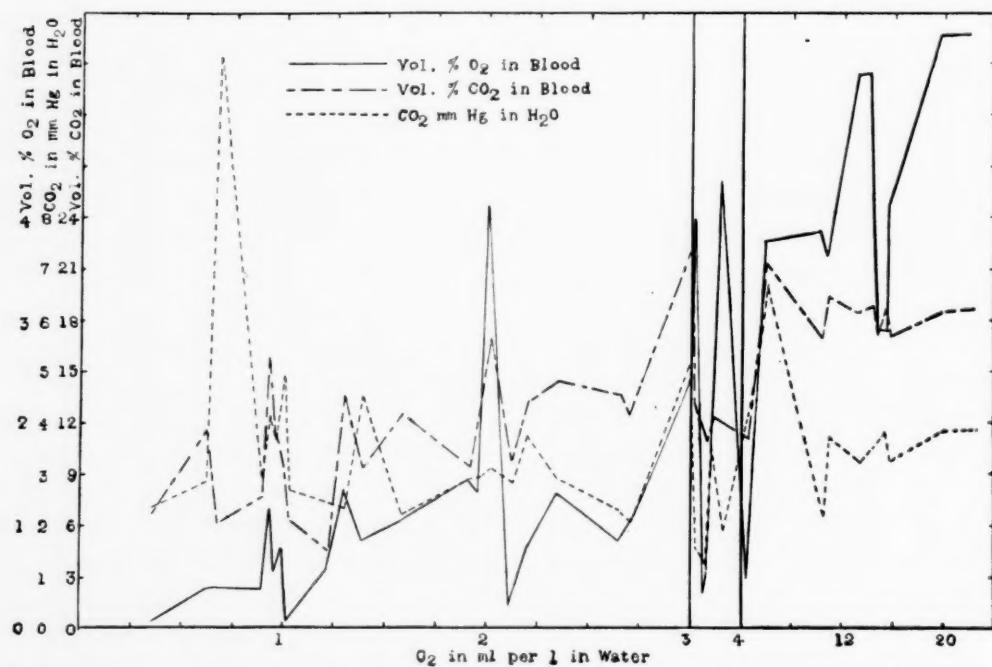


Figure 1 is a graphic representation of data given in Table IV. In this and all subsequent figures, a solid vertical line indicates a change in scale of base.

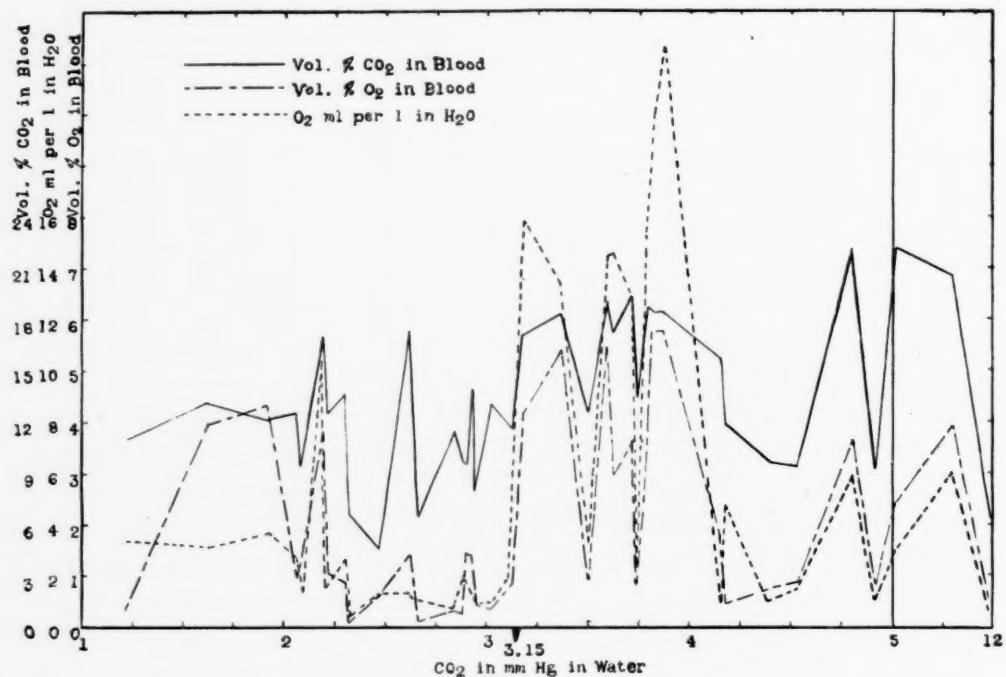


Figure 2 is a graphic representation of data given in Table V.

TABLE V

Same data as in Table IV, except arranged according to an increase in the carbon dioxide tension of the water.

Expt. No.	CO ₂ tension of water in mm Hg	Vol. % CO ₂ in blood	Vol. % O ₂ in blood	O ₂ in ml per 1 in water
1	1.22	10.93	0.36	3.32
39	1.62	13.19	3.97	3.10
40	1.92	12.17	4.35	3.60
4	2.06	12.43	1.00	2.70
2	2.08	9.33	1.36	1.36
21	2.19	17.47	4.00	10.20
22	2.19	16.42	3.73	10.42
3	2.21	12.49	1.03	1.58
7	2.30	13.73	0.86	2.65
16	2.31	6.64	0.09	0.35
18	2.47	4.54	0.61	1.22
10	2.61	17.25	1.35	1.23
17	2.66	6.44	0.09	1.02
19	2.84	11.52	0.34	0.63
20	2.88	9.75	0.25	2.12
11	2.90	9.59	1.45	1.91
12	2.93	14.59	1.32	2.35
13	2.93	13.22	1.30	1.97
15	2.94	7.64	0.40	0.90
14	3.02	13.10	0.36	0.96
6	3.15	11.65	0.88	2.01
27	3.15	17.04	4.15	15.83
33	3.36	18.38	5.39	13.27
5	3.50	12.46	0.93	3.43
34	3.59	18.82	5.42	14.42
29	3.62	17.16	2.93	14.58
30	3.71	19.48	3.64	12.83
8	3.74	13.34	0.76	2.02
28	3.78	18.68	2.90	15.46
32	3.81	18.48	5.78	19.96
31	3.86	18.49	5.79	22.65
9	4.15	15.76	1.79	0.92
42	4.18	11.85	0.49	4.76
36	4.39	9.66	0.73	0.96
35	4.53	9.37	0.85	1.39
24	4.80	21.99	3.62	5.97
37	4.92	9.32	0.82	1.00
26	5.11	22.13	2.41	3.00
23	8.57	20.65	3.92	5.97
38	11.08	6.06	0.42	0.67

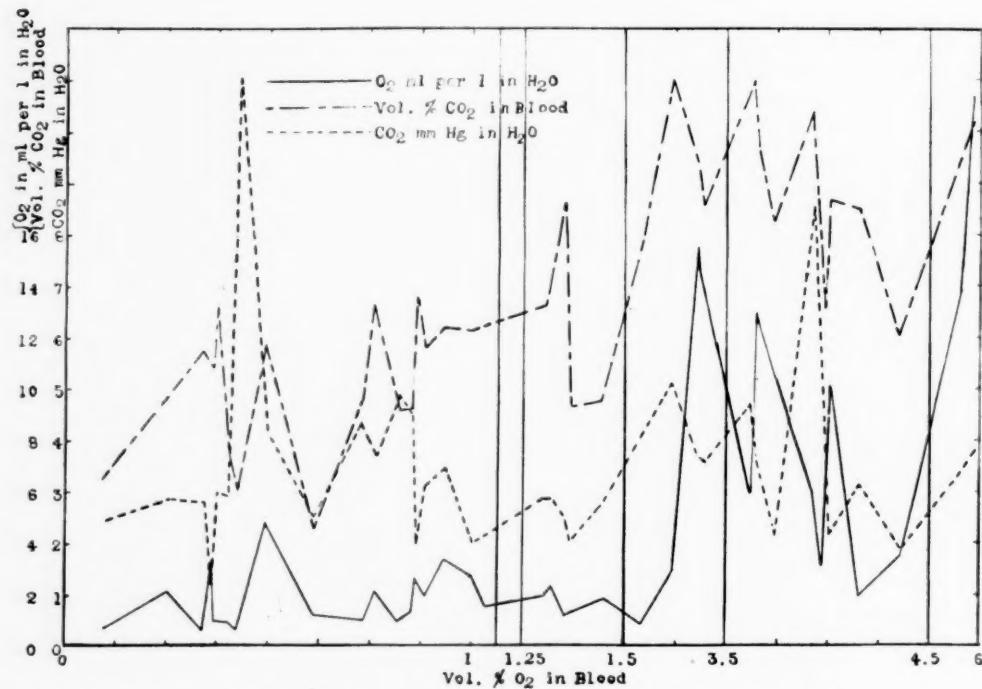


Figure 3 is a graphic representation of data given in Table VI.

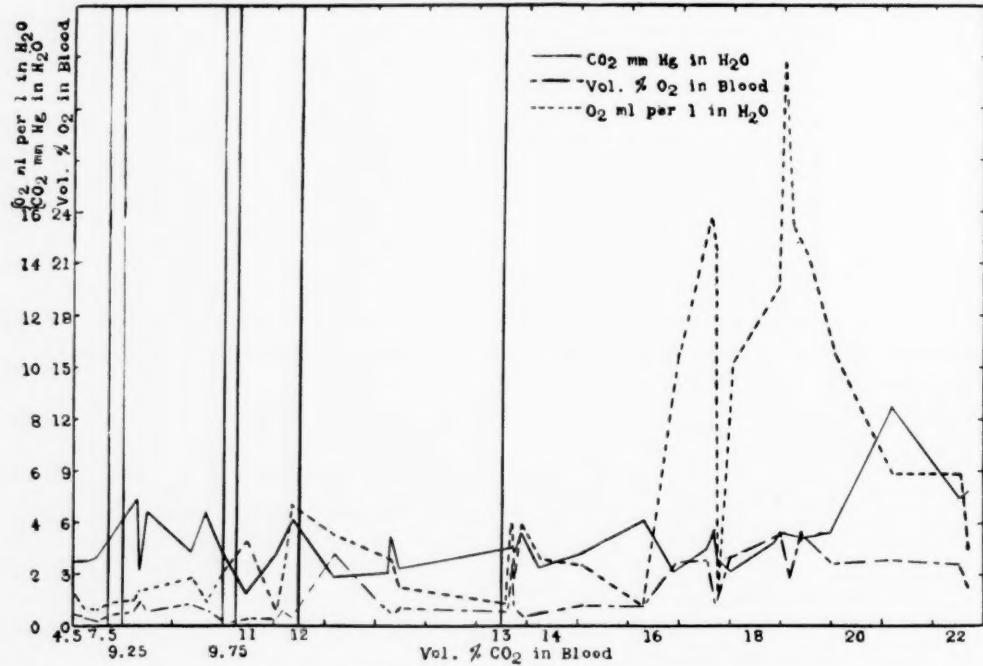


Figure 4 is a graphic representation of data given in Table VII.

TABLE VI

Same data as in Table IV, except arranged according to an increase in the volume per cent of oxygen in the blood.

Expt. No.	Vol. % O ₂ in blood	O ₂ in ml per 1 in water	Vol. % CO ₂ in blood	CO ₂ in mm Hg in water
16}	0.09	0.35	6.64	2.31
17}	0.09	1.02	6.44	2.66
20	0.25	2.12	9.75	2.88
19	0.34	0.63	11.52	2.84
1	0.36	3.32	10.93	1.22
14	0.37	0.96	13.10	3.02
15	0.40	0.90	7.64	2.94
38	0.42	0.67	6.06	11.08
42	0.49	4.76	11.83	4.18
18	0.61	1.21	4.54	2.47
36	0.73	0.96	9.66	4.39
8	0.76	2.02	13.34	3.74
37	0.82	1.00	9.32	4.92
35	0.85	1.39	9.37	4.53
7	0.86	2.65	13.76	2.30
6	0.88	2.01	11.65	3.15
5	0.93	3.43	12.46	3.50
4	1.00	2.70	12.43	2.06
3	1.03	1.58	12.49	2.21
13	1.30	1.97	13.22	2.93
12	1.32	2.35	14.59	2.93
10	1.35	1.23	17.25	2.61
2	1.36	1.36	9.33	2.08
11	1.45	1.91	9.59	2.90
9	1.79	0.92	15.77	4.15
26	2.41	3.00	22.13	5.11
28	2.90	15.46	18.68	3.78
29	2.93	14.58	17.16	3.62
24	3.62	5.97	21.99	4.80
30	3.64	12.82	19.48	3.71
22	3.73	10.42	16.42	2.19
23	3.92	5.97	20.65	8.57
39	3.97	3.10	13.19	1.63
21	4.00	10.20	17.47	2.19
27	4.15	2.01	17.04	3.15
40	4.35	3.60	12.17	1.92
33}	5.39	13.27	18.38	3.26
34}	5.42	13.85	18.82	3.59
32}	5.78	19.96	18.48	3.81
31}	5.79	21.30	18.48	3.83
		22.65	18.49	3.86

TABLE VII

Same data as in Table IV, except arranged according to an increase in the volume per cent of carbon dioxide in the blood.

Expt. No.	Vol. % CO ₂ in blood	CO ₂ in mm Hg in water	Vol. % O ₂ in blood	O ₂ in ml per 1 in water
18	4.54	2.47	0.61	1.21
38	6.06	2.47	0.42	0.67
16}	6.64	2.31	0.09	0.35
17}	6.44	2.49	0.09	1.02
	6.54	2.66	0.09	0.64
15	7.64	2.94	0.40	0.90
37	9.32	4.92	0.82	1.00
2	9.33	2.08	1.36	1.36
35	9.37	4.53	0.85	1.39
11	9.59	2.90	1.45	1.91
36	9.66	4.39	0.73	0.96
20	9.75	2.88	0.25	2.12
1	10.93	1.22	0.36	3.32
19	11.52	2.84	0.34	0.63
6	11.65	3.15	0.88	2.01
42	11.82	4.18	0.49	4.76
40	12.17	1.92	4.35	3.60
4	12.43	2.06	1.00	2.70
5	12.46	3.50	0.93	3.43
3	12.49	2.21	1.03	1.58
14	13.10	3.02	0.36	0.96
39	13.19	3.10	3.97	2.97
13	13.22	2.93	1.30	1.97
8	13.34	3.74	0.76	2.02
7	13.73	2.30	0.86	2.65
12	14.59	2.93	1.32	2.35
9	15.77	4.15	1.18	0.92
22	16.42	2.19	3.73	10.42
27	17.04	3.15	4.15	15.83
29	17.16	3.62	2.93	14.58
10	17.25	2.61	1.35	1.23
21	17.42	2.19	4.00	10.20
33	18.38	3.26	5.39	13.27
32}	18.48	3.81	5.78	19.96
31}	18.49	3.86	5.79	22.65
	18.48	3.83	5.78	21.80
28	18.68	3.78	2.90	15.46
34	18.82	3.59	5.42	14.42
30	19.48	3.71	3.64	10.82
23	20.65	8.57	3.92	5.97
24	21.99	4.80	3.62	5.97
26	22.13	5.11	2.41	3.00

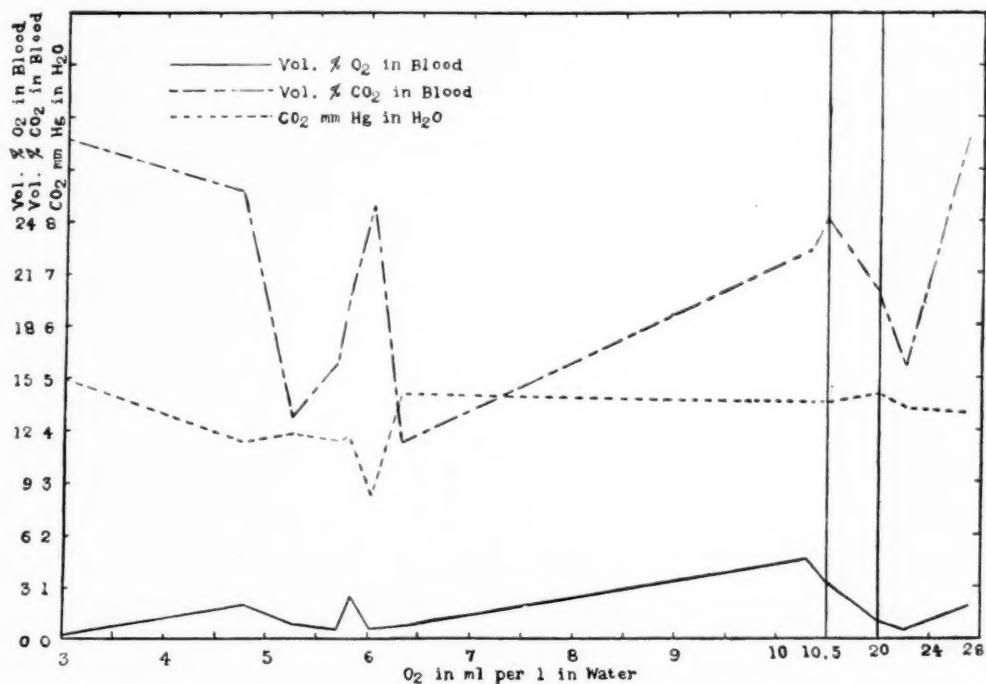


Figure 5 is a graphic representation of data given in Table VIII.

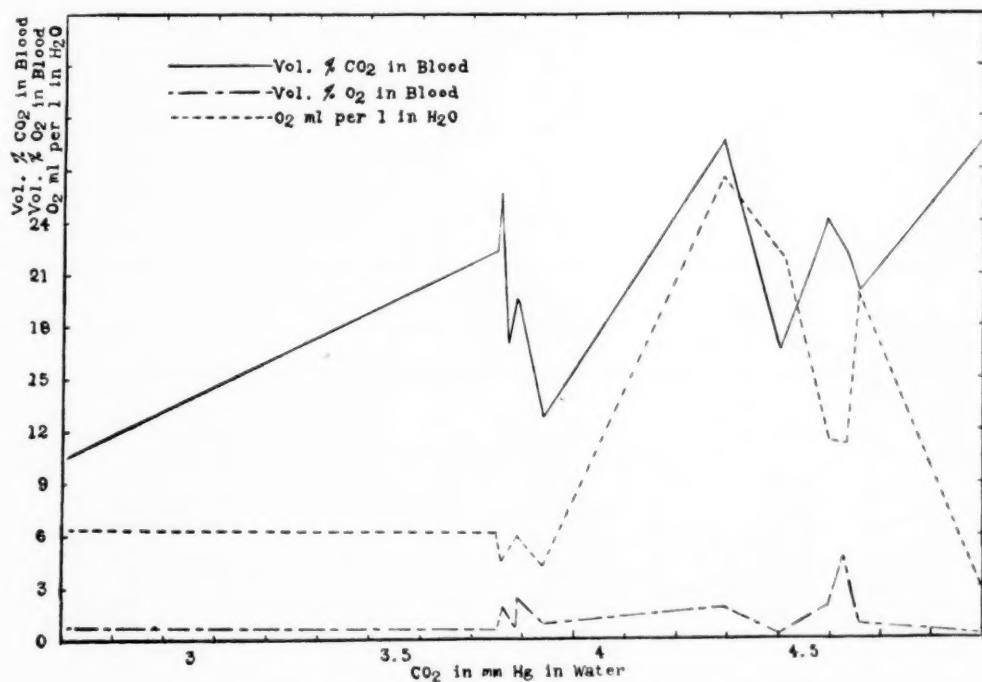


Figure 6 is a graphic representation of data given in Table IX.

TABLE VIII

Data showing the interrelations between the oxygen content and carbon dioxide tension of the water and the oxygen and carbon dioxide volume per cents of the blood of the German carp, *Cyprinus carpio*, Linneaus, when arranged according to an increase in the oxygen content of the water.

Expt. No.	O ₂ in ml per 1 of water	Vol. % of O ₂ in blood	Vol. % of CO ₂ in blood	CO ₂ in mm of Hg in water	Temperature, °C.
60	3.01	0.40	28.6	4.93	28
44	4.76	1.94	25.6	3.76	26
45	5.25	1.00	12.7	3.91	26
46	5.68	0.62	15.8	3.79	26
49	5.82	2.51	19.6	3.80	27
51	6.03	0.62	25.1	3.75	27
47	6.33	0.89	10.60	2.70	29
53	10.28	4.59	22.30	4.60	27
54	10.49	3.28	24.20	4.54	27
56	20.09	0.66	20.00	4.63	22
55	22.08	0.56	15.60	4.44	22
57	26.48	1.81	28.70	4.30	26

TABLE IX

Same data as in Table VIII, except arranged according to an increase in the carbon dioxide tension of the water.

Expt. No.	CO ₂ in mm Hg in water	Vol. % CO ₂ in blood	Vol. % O ₂ in blood	O ₂ in mm per 1 in water
47	2.70	10.60	0.89	6.33
51	3.75	25.10	0.62	6.03
44	3.76	25.60	1.94	4.76
46	3.79	15.80	0.62	5.68
49	3.80	19.60	2.51	5.82
45	3.91	12.70	1.00	5.25
57	4.30	28.70	1.81	26.48
55	4.44	15.60	0.56	22.08
54	4.56	24.20	3.28	10.49
53	4.60	22.30	4.59	10.28
56	4.63	20.00	0.66	20.09
60	4.93	28.60	0.40	3.01

TABLE X

Same data as in Table VIII, except arranged according to an increase in the volume per cent of oxygen in the blood.

Expt. No.	Vol. % O ₂ in blood	O ₂ in ml per 1 in water	Vol. % CO ₂ in blood	CO ₂ in mm Hg in water
60	0.40	3.01	28.60	4.93
55	0.56	22.08	15.60	4.44
51	0.62	6.03	25.10	3.75
46	0.62	5.68	15.80	3.79
56	0.66	20.09	20.00	4.63
47	0.89	6.33	10.60	2.70
45	1.00	5.25	12.70	3.91
57	1.81	26.48	28.70	4.30
44	1.94	4.76	25.60	3.76
49	2.57	5.82	19.60	3.80
54	3.28	10.49	24.20	4.56
53	4.59	10.28	22.30	4.60

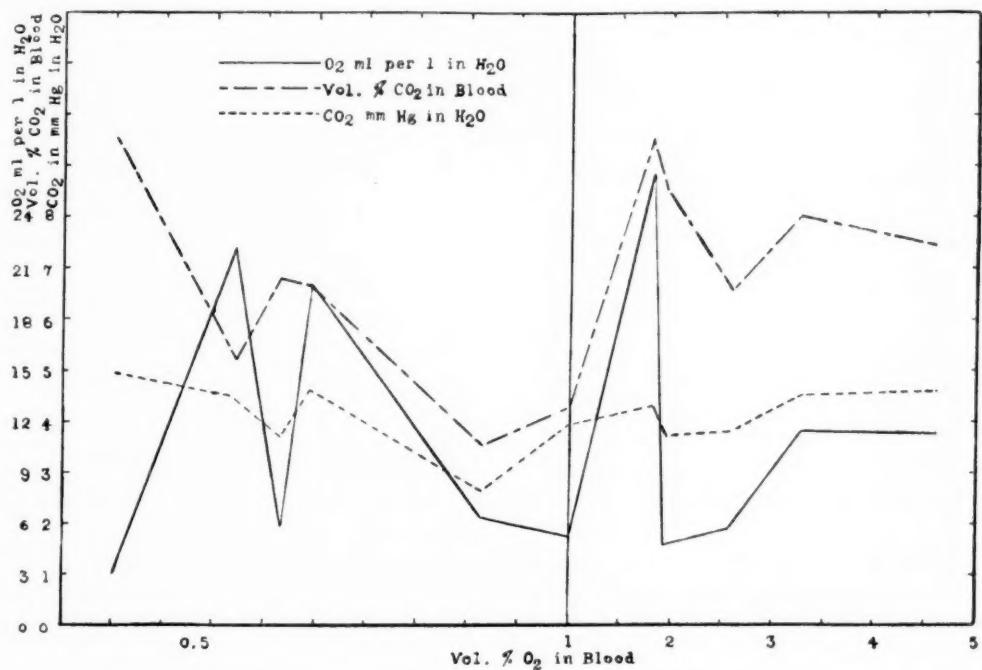


Figure 7 is a graphic representation of data given in Table X.

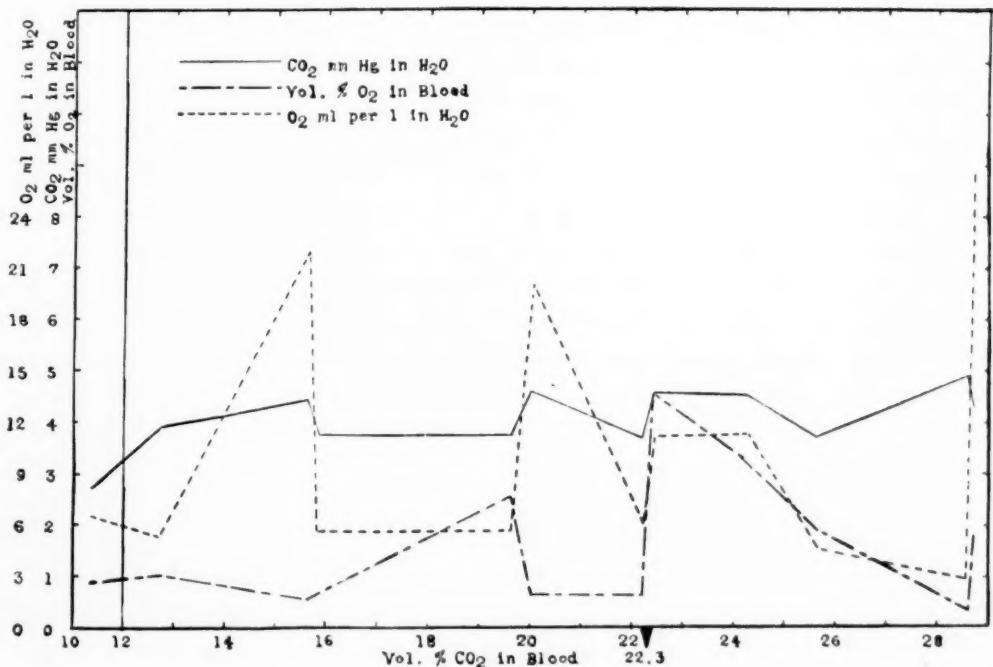


Figure 8 is a graphic representation of data given in Table XI.

TABLE XI

Same data as in Table VIII, except arranged according to an increase in the volume per cent of carbon dioxide in the blood.

Expt. No.	Vol. % CO ₂ in blood	CO ₂ in mm Hg in water	Vol. % O ₂ in blood	O ₂ in mm per 1 in water
47	10.60	2.70	0.89	6.33
45	12.70	3.91	1.00	5.25
55	15.60	4.44	0.56	22.08
46	15.80	3.79	0.62	5.68
49	19.60	3.80	2.57	2.82
56	20.00	4.63	0.66	20.09
53	22.30	4.60	4.59	10.28
54	24.20	4.56	3.28	10.49
51	25.10	3.75	0.62	6.03
44	25.60	3.76	1.94	4.76
60	28.60	4.93	0.40	3.01
57	28.70	4.30	1.81	26.48

V. EFFECT OF THE OXYGEN AND CARBON DIOXIDE TENSIONS
OF THE WATER UPON THE NUMBER OF RED COR-
PUSCLES IN THE BLOOD OF THE BLUE CAT,
ICTALURUS PUNCTATUS (RAFINESQUE)

BY EDWIN B. POWERS AND LULA MAE SHIPE

INTRODUCTION

At the same time that blood was being drawn for oxygen and carbon dioxide determinations in the preceding work, samples were taken for red blood corpuscle counts. The gas content of the swim-bladder was also taken at the same time, this to be used in working out the mechanism of oxygen deposition into the swim-bladder. It was our idea that by making all these tests at the same time on the same fish, it would eliminate to a great extent individual variations in changes taking place in the composition and gas contents of the blood in response to oxygen and carbon dioxide tensions of the water, *i.e.*, the external environment. The attempt to determine the responses in so many factors in the same fish militated somewhat against our always getting a sample of blood representative of the individual fish while it was under the conditions of the experiment. When this could not be done no count of corpuscles was made.

METHOD AND MATERIAL

The Thoma-Zeiss haemacytometer equipped with a Levy counting chamber, having Neubauer's improved ruling, was used, and the method suggested by Stitt (1927) was followed in counting the red blood corpuscles. With the exception of the first four experiments, the blood was taken directly from the ventricle of the heart and a sample was immediately drawn up into a di-

luting pipette. Hayem's solution was used as the diluting fluid. This was all done before the blood had time to clot.

The blue cat, *Ictalurus punctatus* (Rafinesques), was used in all experiments. The blood was taken from fishes used in the preceding work, and the same number has been used for the same experiment in both instances.

EXPERIMENTAL DATA

Table XII gives the number of red blood corpuscles in millions and fraction thereof per cubic mm of blood. The data are arranged in the order of increase in oxygen content of the water in which the fish was placed. Column three gives the carbon dioxide tension of the water. Figure 9 is a graphic representation of the data of Table XII. When the graph is examined it is noted that the number of red corpuscles in the blood tends to decrease with an increase in the oxygen content of the water and that there is a marked parallelism between the lines representing the number of red blood corpuscles and the carbon dioxide tension of the water.

In order to see whether or not the number of red blood corpuscles was modified by the carbon dioxide tension of the water, the data were arranged in the order of increase in the carbon dioxide tension of the water. Table XIII, column three, gives the oxygen content of the water. Figure 10 is a graphic representation of the data given in Table XIII. The oxygen content of the water has been drawn in the reverse order, i.e., the top of the graph represents low oxygen content of the water and the bottom high oxygen content. The reason for this reversed arrangement of the oxygen content of the water is that low oxygen tension of the blood causes an increase in the number of red corpuscles in the blood of all mammals thus far tested (see Barcroft, 1925 and citations). The line representing the number of red blood corpuscles was so placed on the graph that it approximates the same position as the line representing the reverse order of the oxygen content of the water. This was done so that the two could be more easily compared.

When the graph (Figure 10) is examined, two things are obvious. First, there is a trend for the red blood corpuscles to increase with an increase in the carbon dioxide tension of the water. Second, there is a marked parallelism between the lines representing the number of red blood corpuscles and the reverse order of the oxygen content of the waters.

From Tables XII and XIII and Figures 9 and 10, it is obvious that the number of red blood corpuscles is increased with a decrease in the oxygen content of the water and is decreased with an increase in the oxygen content, and also that the number of red blood corpuscles is increased with an increase in the carbon dioxide tension of the water and *vice versa*. The effect of the oxygen content and the carbon dioxide tension of the water upon the number of red blood corpuscles seems to be independent of each other. In order to

test these conclusions, the data were arranged in order of increase in number of red blood corpuscles, Table XIV, and plotted against the oxygen contents and carbon dioxide tensions of the water, Figure 11.

Figure 11 shows three things. First, the lines representing the oxygen contents and the carbon dioxide tensions of the water show the various combinations of oxygen and carbon dioxide tensions of the experimental waters. Second, the trend of the line representing the oxygen content of the water shows that the number of red blood corpuscles is increased with a decrease in the oxygen content of the water and *vice versa*. Third, it shows that the carbon dioxide tension of the water has just the reverse effect.

There are marked exceptions to the conclusions that the oxygen content and the carbon dioxide tension of the water have reverse effects upon the red blood corpuscles. The exceptions are fewer than the rule. The exceptions are not surprising to those who are accustomed to experimental work with live animals when the individual variations, the variations in the immediate previous history of the fish, the variation in the duration of the experiments and the different degrees of adjustment of the fish to the conditions of the experimental water at the termination of an experiment are considered. These were all too complicated to control with the facilities at hand. Such conditions as individual variations are far beyond the control of any laboratory technique. Table VIII gives the time duration of the experiments and a few notes on the conditions of a few individual fishes at the close of the experiments.

DISCUSSION

It has been known since the time of Paul Bert that the number of red blood corpuscles was increased with altitude. This was worked out in detail during the Pike's Peak Expedition. This and all subsequent works led the workers to attribute this increase in red blood corpuscles to lowering of the oxygen pressure with the lowering of the barometric pressure (see Haldane, 1922, and Barcroft, 1925, for discussions and citations). Campbell (1927) has shown that this effect is due to the oxygen pressure independent of barometric pressure. Hall (1928) and Hall, Gray, and Lepkovsky (1926) have shown that there is an increase in the number of red corpuscles with asphyxiation of fishes and that the increase is roughly proportional to the time of asphyxiation and have thus put the fishes in the same category with mammals in this respect.

As far as we are aware, no one has before shown that the carbon dioxide tension has a direct effect upon the number of red blood corpuscles of fish blood. However, Brednow (1930) has shown that breathing high carbon dioxide percentages calls forth an increase in red corpuscles in the circulating plasma of man. The advantage of such a mechanism among fishes is quite obvious. A fish gives off carbon dioxide from the tissues in the same pro-

portion as it utilizes oxygen. This oxygen must be supplied by the oxyhemoglobin being changed to hemoglobin. The amount of oxygen given off depends upon the changes in the blood brought about by the addition of carbon dioxide by the tissues, the amount of hemoglobin in the blood, the rate of blood supply, and the oxygen tension at which the oxygen is removed from the capillaries. This is supposing that the blood is loaded with oxygen to its full capacity as it passes through the capillaries of the gills. When the carbon dioxide tension of the water is increased, the active alkalinity of the blood is increased (p. 396). With a high active alkalinity in the blood and everything else being equal, such as the loading of oxygen in the gills, the rate of blood supply, the amount of hemoglobin in the blood, the oxygen tension at which the oxygen leaves the capillaries, a given amount of carbon dioxide given up to the blood by the tissues will not call forth the liberation of as much oxygen as when there is a low active alkalinity in the blood. This is true whether the liberation of oxygen from the oxyhemoglobin as brought about by the addition of the carbon dioxide to the blood is due to the change in the hydrogen ion concentration of the blood plasma or is due to the changes that take place within the red blood corpuscle by the diffusion of the carbon dioxide itself into the corpuscle. In the first place, the blood with a high active alkalinity will not undergo as great a change in hydrogen ion concentration with a given amount of carbon dioxide as when there is a low active alkalinity. This is the fundamental principle elucidated by L. J. Henderson (1908) and upon which the vast knowledge of the chemistry of the blood has been built. In the second place, if the liberation of oxygen by the oxyhemoglobin is brought about by the changes within the corpuscle by the diffusion of the carbon dioxide as such, the diffusion tension would not be increased by the given amount of carbon dioxide in blood with a high active alkalinity as in blood with a low active alkalinity. This again is the fundamental principle of L. J. Henderson's (1908) acid-base equilibrium. A part of the carbon dioxide would be tied up by the active alkalinity of the blood and thus leave less carbon dioxide to increase the carbon dioxide tension of the blood. In either or both cases the advantage of increasing the oxygen carrying capacity of the blood is obvious. This will allow more oxygen to be removed from the capillaries at a given tension, other things being equal. Furthermore, blood will not load as high a per cent in the capillaries of the gills at a high carbon dioxide tension as at a low carbon dioxide tension (Bohr, 1904, and all subsequent workers) unless the blood has been modified. This sets forth another obvious advantage in the increase in the number of red blood corpuscles with an increase in the carbon dioxide tension of the water bathing the gills of fishes. It remains to be determined what the actual stimulus is that calls forth an increase in or diminishes the number of red corpuscles in the blood of either fishes or mammals.

TABLE XII

Data showing the behavior of the red blood corpuscles of the blue cat, *Ictalurus punctatus* (Rafinesque), in response to oxygen content (tension) and carbon dioxide tension of the water are arranged according to increase in oxygen content of the water.

Expt. No.	O ₂ ml per 1 in H ₂ O	Red corpuscles in millions per mm ³	CO ₂ in mm Hg in H ₂ O
16	0.35	2.085	2.31
19	0.63	2.855	2.84
15	0.90	2.360	2.94
9	0.93	3.505	4.15
14	0.96	2.055	3.02
17	1.02	2.660	2.66
10	1.24	2.310	2.61
2	1.35	2.020	2.08
3	1.58	2.045	2.21
11	1.91	2.510	2.90
20	2.12	3.565	2.88
8	2.20	4.290	3.74
12	2.35	3.040	2.93
26	3.00	3.315	5.11
1	3.32	2.490	1.22
5	3.43	2.951	3.50
23	5.97	1.965	8.57
24	5.97	2.865	4.80
21	10.20	2.350	2.19
22	10.42	1.240	2.19
30	10.82	1.410	3.11
29	14.58	2.865	3.62
28	15.46	2.795	3.78
27	15.83	1.660	2.15
32	19.96	2.450	3.81
31	22.65	1.990	3.85

TABLE XIII

The same data as in Table XII are arranged according to an increase in carbon dioxide tension of the water.

Expt. No.	CO ₂ mm Hg in H ₂ O	Red corpuscles in millions per mm ³	O ₂ ml per 1 in H ₂ O
1	1.22	2.490	3.32
2	2.08	2.020	1.36
21	2.19	2.450	10.20
22	2.19	1.240	10.40
3	2.21	2.045	1.58
16	2.31	2.085	0.35
10	2.61	2.310	1.23
17	2.66	2.660	1.02
19	2.84	2.855	0.63
20	2.88	3.565	2.12
11	2.90	2.510	1.91
12	2.93	2.040	2.35
15	2.94	2.360	0.90
14	3.02	2.055	0.96
27	2.15	1.600	15.83
5	3.50	2.950	3.43
29	3.62	2.865	14.58
30	2.71	1.410	12.83
8	3.74	4.290	2.02
28	3.78	2.795	15.46
32	3.81	2.540	19.96
31	3.86	1.990	22.65
9	4.15	3.505	0.92
24	4.80	2.865	5.97
26	5.11	3.315	3.00
23	8.57	1.965	5.97

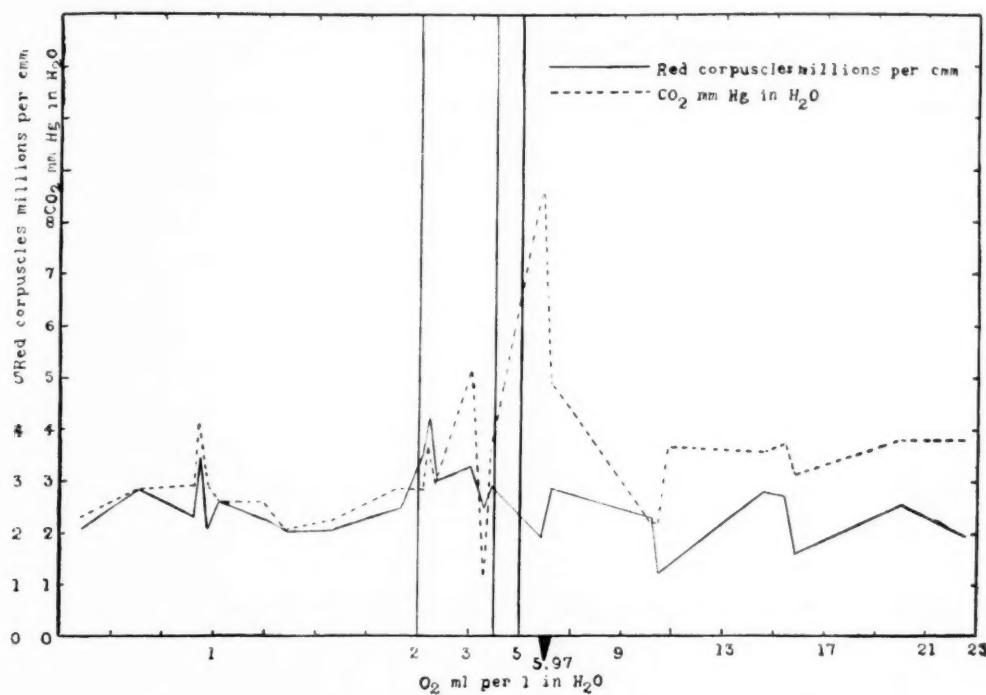


Figure 9 is a graphic representation of data given in Table XII.

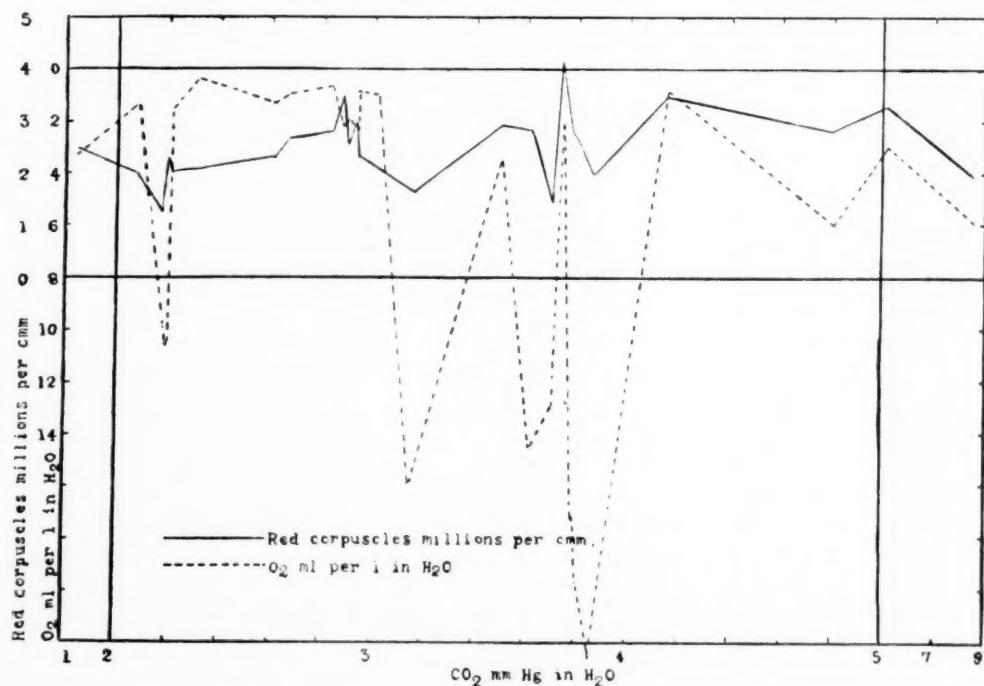


Figure 10 is a graphic representation of data given in Table XIII.
The oxygen content of the water is plotted in reverse order.

TABLE XIV

The same data as in Tables XII and XIII are arranged according to an increase in the number of red blood corpuscles.

Expt. No.	Red corpuscles in million per mm ³	O ₂ ml per 1 in H ₂ O	CO ₂ mm Hg in H ₂ O
22	1.240	10.42	2.19
30	1.410	10.82	3.71
27	1.660	19.96	3.15
23	1.965	5.97	2.19
31	1.990	22.65	3.85
2	2.020	1.35	2.08
3	2.045	1.58	2.21
14	2.055	0.96	3.02
16	2.085	0.35	2.31
10	2.310	1.24	2.61
21	2.350	10.20	2.19
15	2.360	0.90	2.94
1	2.490	3.32	1.22
11	2.510	1.91	2.90
32	2.540	19.96	3.81
17	2.660	1.02	2.66
28	2.795	15.46	3.78
19	2.855	0.63	2.84
24	2.865	5.97	4.80
29	2.865	14.58	3.62
5	2.951	3.43	3.50
12	3.040	2.35	2.93
26	3.315	3.00	5.11
9	3.505	0.93	4.15
20	3.565	2.12	2.88
8	4.290	2.20	3.74

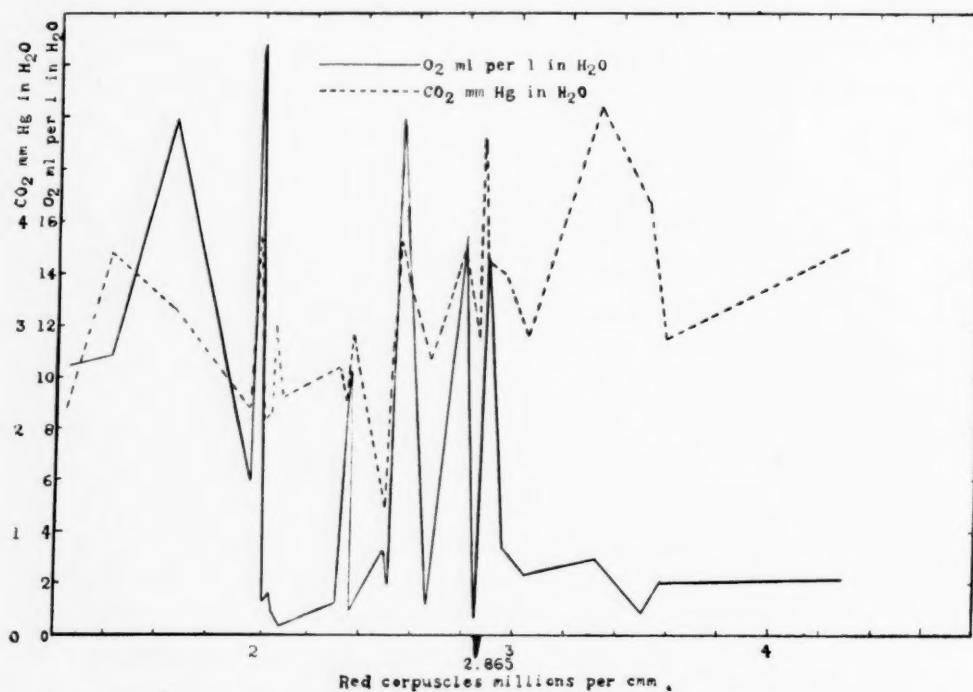


Figure 11 is a graphic representation of data given in Table XIV.

VI. OXYGEN AND CARBON DIOXIDE DISSOCIATION CURVES OF WHOLE BLOOD

BY EDWIN B. POWERS AND THRESSA A. HICKMAN

Fishes live in a medium in which the oxygen and carbon dioxide tensions approximate the oxygen and carbon dioxide partial pressures of the atmosphere. It then becomes desirable to know the oxygen carrying capacities of fish bloods at very low carbon dioxide tensions and how their capacities are affected by a change in either the oxygen or the carbon dioxide tensions of the bloods. Krogh and Leitch (1919) determined the oxygen dissociation curve without the presence of carbon dioxide. They found that carp's blood was half saturated at very low pressures (2-3 mm at 15° C) of oxygen and that the blood of the cod, plaice, and the trout were half saturated at 10-11 mm of oxygen. Later Wastl (1928) determined the oxygen dissociation curve of the carp's blood over a wider range of oxygen tensions. Both Krogh and Leitch and Wastl noted that the loading oxygen tensions were much higher even when very small amounts of carbon dioxide were present. Kawamoto (1929a) also determined oxygen dissociation curves of fish blood. By comparison of the oxygen dissociation curves of the eel's blood, he found Q_{10} to be 1.56 at 5° to 30° C.

In the experiments to be described the oxygen dissociation curves were first determined at low carbon dioxide tension. Later the work was extended to include the carbon dioxide dissociation curve and also the effect of various carbon dioxide tensions upon the oxygen capacities of the blood equilibrated at a given oxygen tension.

The blood was always drawn directly from the heart or the conus arteriosus. A mixture of potassium oxalate and ammonium fluoride was used as an anticoagulant. The blood was placed in cold storage immediately after being drawn. When sufficient amounts of blood were obtained, one ml samples were used, otherwise 0.2 ml samples were resorted to in determining the gases of the blood (Van Slyke and Neil, 1924, Harrington and Van Slyke, 1924, and Van Slyke, 1927). The technique in handling fish blood is very difficult. This is due to the rapid changes taking place in the blood, the causes of which will be discussed in the following pages. On the other hand the technique in handling fish blood is simplified by the fact that the partial pressures of oxygen and carbon dioxide of the atmosphere approximate the oxygen and carbon dioxide tensions of the water used by the fishes to bathe their gills in the aeration of their blood. Thus it was not essential to take the precaution necessary to prevent the blood from coming in contact with the air. As the carbon dioxide partial pressures were increased for equilibration, which as we will point out later is one of the factors bringing about modifications in the blood, it became difficult to obtain checks in successive duplicate carbon dioxide and oxygen determinations of the blood. At the uppermost

carbon dioxide partial pressure range used in our experiments it was extremely difficult to obtain absolute checks. Two people operating Van Slyke apparatus simultaneously were able to get better checks on the oxygen and carbon dioxide determinations than either was able to check himself in a second determination. Good checks were obtained at very low carbon dioxide tensions of the blood.

DISCUSSION OF EXPERIMENTAL DATA

Blood was taken from the ventricle and conus arteriosus of a thirty pound yellow cat immediately after the fish had been taken from a live-box in the river in which it had been trapped the previous day. The blood was immediately packed in ice, carried to the laboratory and placed in cold storage. In all other cases to be described in this paper, the blood was taken from the fish in the laboratory and immediately placed in cold storage. These fishes were kept in an artificial pool for at least twenty-four hours, which enabled them to recover at least in part from the shock of transportation to the laboratory.

The blood taken from the yellow cat, *Leptops olivaris* (Rafinesque), was equilibrated in a tonometer at room temperature and barometric pressure with oxygen to which had been added percentages of carbon dioxide from .015% to 9.5% and the volume per cents of oxygen and carbon dioxide determined with the Van Slyke Gas Analysis Apparatus. See Table XV and Figure 12. The higher carbon dioxide percentages were used in order to determine the behavior of the blood in contact with carbon dioxide percentages normally found in the swim-bladder of fishes inhabiting shallow waters. An examination of the carbon dioxide dissociation curve, Figure 12, shows that the carbon dioxide volume per cent rises at first rapidly, up to about .5% carbon dioxide, and then less rapidly up to 9.5%, the highest carbon dioxide tension tested, with increase in carbon dioxide.

When the curve representing the oxygen volume per cent of the blood at the different carbon dioxide tensions tested is examined, it is found that there is a rapid falling off of the oxygen content of the blood at very low carbon dioxide percentages with increase of carbon dioxide used in equilibration. This was followed by a rise in the oxygen volume per cent reaching its highest point at 0.6% carbon dioxide according to our data. After this there was a decline, the oxygen volume per cent falling off with each additional increase in carbon dioxide per cent.

Two oxygen dissociation curves were then determined, one at approximately 0.037% carbon dioxide and from 2% to approximately 100% oxygen and the other at approximately 2% carbon dioxide and from 8.4% to 97.9% oxygen at room temperature and barometric pressure.

When the two curves are plotted and compared, Table XVI and Figure 13, it is seen that they are related to each other as two entirely different

bloods. The shapes of the two curves indicate that the blood when equilibrated with 2% carbon dioxide is modified so as not to have as great a capacity for oxygen as the blood does when equilibrated with .037% carbon dioxide.

Next a carbon dioxide dissociation curve was determined on the blood of a four to five pound black horse, *Cycleptus elongatus* (Le Sueur). When this carbon dioxide dissociation curve is examined, Figure 14 and Table XVII, it is seen that it is not a smooth curve but that there is a concavity at .22% carbon dioxide, that is, between .1% and .41% carbon dioxide. As in the blood of the yellow cat, with the exception just mentioned, there is at first a rapid rise in the carbon dioxide content of the blood, followed by a less rapid rise with an increase in the carbon dioxide tension.

The oxygen content of the blood equilibrated with approximately 20% oxygen, Figure 14, first fell, followed by a slight rise which in turn was followed by a somewhat rapid gradual lowering with an increase in the carbon dioxide tension. This fall in the oxygen content of the black horse blood with rise in carbon dioxide tension was more rapid than in the yellow cat.

The blood of a nine pound German carp, *Cyprinus carpio* Linneaus was then taken. Great care was exercised in handling the carp blood. It seems to go to pieces at the least provocation. Before all the necessary determinations could be made the blood had changed to a dark cherry red color and was discarded.

On two other occasions carp were obtained, one weighing fourteen pounds and the other twenty. Both times the carp blood went to pieces rapidly. In the first case samples of blood were equilibrated and determinations made for oxygen and carbon dioxide contents. We failed entirely to obtain any kind of checks. At the higher carbon dioxide percentages small gelatinous clots adhered to the sides of the tonometer when rotated. The data were so inconsistent that all were discarded. In the case of the second carp everything was made ready before the blood was drawn. Despite the fact that the blood was drawn very carefully, it had turned a very dark wine color which persisted and gelatinous clumps adhered to the sides of the tonometer when the blood was being equilibrated. Further determinations were not attempted and no further efforts were made to use carp blood to determine dissociation curves.

Since we were not able to use the carp, we obtained an eighteen pound blue cat, *Ictalurus punctatus* (Rafinesque), to confirm our results. A carbon dioxide dissociation curve was determined with oxygen at approximately 20%, Table XVIII and Figure 15. The carbon dioxide dissociation curve was slightly irregular, again showing the rapid rise in carbon dioxide content with increase in carbon dioxide tension at the low tensions followed by a less

rapid somewhat irregular rise. The curve representing the oxygen content has the same general form as those of the yellow cat and the black horse.

For further confirmation a twenty-two pound yellow cat was then obtained and a carbon dioxide dissociation curve was determined at low carbon dioxide tensions with very slight variations in carbon dioxide percentages used for equilibration, Figure 16 and Table XIX. Again there was a rapid rise in carbon dioxide contents of the blood at low carbon dioxide tensions when equilibrated with small amounts, 0.015 to .24%, of carbon dioxide. This again was followed by a less rapid but irregular rise.

The curve representing the oxygen contents of the blood at the different carbon dioxide tensions again, as in the three preceding curves, shows at first a depression followed by a slight rise which again was followed by a less rapid gradual depression.

DISCUSSION

In these experiments the oxygen dissociation curves of the blood of the yellow cat follow the general form of the oxygen dissociation curves determined by Bohr (1904) and all subsequent workers. There was no attempt made to determine whether it conforms more nearly with the S-form of curve or a parabola. The graphs are free hand drawings. No attempt was made to draw them so as to conform with any mathematical formula. The purpose of this investigation was rather to determine the behavior of fish blood when equilibrated with different percentages of carbon dioxide.

The two oxygen dissociation curves, one with .03% and the other with 2% carbon dioxide, differ from each other as would two entirely different bloods. These two curves show relations to each other as do the oxygen dissociation curves when determined: first, on two entirely different bloods; second, on bloods of the same individual under different environmental conditions; and third, on two samples of the same blood, one sample having had red corpuscles extracted from it and the other having had red corpuscles added (Barcroft, 1925, and citations). In other words the two oxygen dissociation curves of the blood of the yellow cat, one at .03% and the other at 2%, differ from each other as would two different bloods having different percentages of hemoglobin content. Thus it seems that the hemoglobin has been changed either in kind or in content.

Rona and Ylppö (1916) were first to find in mammalian blood that the hemoglobin has the least affinity for oxygen at its isoelectric point and that its affinity for oxygen increases both with an increase and a decrease in the hydrogen ion concentration from this point. Pantin and Hogben (1925), Hogben (1926), Hogben and Pinhey (1926, 1927) and Stedman and Stedman (1926, 1926a, and 1927) have found the oxygen transporting pigment of the bloods of invertebrates to have the same characteristics. The curves, however, on the two sides of the isoelectric point differ from each other.

Hogben and Pinhey (1927) state, "In all cases in which this phenomenon has been investigated by the colorimetric method there is a difference in shape of the dissociation curves on either side of the critical pH value indicating a different type of reaction."

The hydrogen ion concentrations of the blood equilibrated with 0.03% carbon dioxide must be on opposite sides of the isoelectric point of the blood. This is indicated by the curves of the oxygen content of the blood at a given oxygen tension at various carbon dioxide tensions. When Figures 12, 14, 15, and 16 are examined it is found that there is at first a depression in the oxygen content of the blood up to about .35% carbon dioxide then there is a rise which is soon followed by a depression. The second depression is perhaps due to the actual breaking down of the hemoglobin into its intermediate products. After this series of papers had been prepared for publication a paper by Root (1931) has come off the press in which the respiratory function of the blood of marine fishes is discussed. Root found this same loss in the capacity of fish blood to combine with oxygen at carbon dioxide tensions higher than the carbon dioxide partial pressure of the atmosphere. He suggests that carbon dioxide at higher tensions inactivates certain of the prosthetic groups concerned in binding oxygen in the hemoglobin molecule. This possibility, that is, the acid effect, has also been suggested by Redfield and Mason (1928) in the case of purified *Limulus* hemocyanin. It is possible that the carbon dioxide effect is due to an inactivation. Experiments to be described later indicate that the changes taking place in the hemoglobin of the fish blood are reversible. However, evidence will be presented in following pages that the hemoglobin is actually being broken down or at least modified by the action of carbon dioxide perhaps acting as an acid.

The pH of the blood without loss of gases was not determined. However, judging from other pH determinations made, probably the pH of fish blood is fairly uniform. If the carbon dioxide tension of fish arterial blood approximates equilibrium with the carbon dioxide partial pressure of the atmosphere the blood would pass through approximately one pH unit when equilibrated with .35% carbon dioxide which is ten times that of the atmosphere. The logarithm of ten is one. All formulas [Hasselbalch (1916), Peters (1923), Saunders (1926), Powers and Bond (1927, 1928), and Powers (1930)] from which pH values of solutions can be calculated are logarithmic. The blood when equilibrated with 3.5% carbon dioxide would for the same reason have passed through approximately two pH units, since 3.5% is one hundred times as great as .035%.

We wish to thank Dr. J. W. Love for assisting in some of the experiments.

TABLE XV

Data show the oxygen and carbon dioxide contents of the blood of the yellow cat, *Leptops olivaris* (Rafinesque), when equilibrated with oxygen to which has been added different percentages of carbon dioxide. Data are arranged both chronologically and in order of increase in carbon dioxide.

Per cent CO ₂ in O ₂ equilibrated with	Volume per cent CO ₂ in blood	Volume per cent O ₂ in blood	Temperature, °C
.015	2.22	11.66	23
.037	2.28	11.17	24
.38	4.80	11.46	24
.6	5.12	11.70	23
2.1	9.40	11.20	20
4.2	15.42	7.70	20
9.5	24.72	8.78	23

TABLE XVI

Data show the oxygen contents of the blood of the yellow cat, *Leptops olivaris* (Rafinesque), when equilibrated with different percentages of oxygen and approximately .03% and approximately 2% carbon dioxide. 24°C.

Approximately .03% carbon dioxide		Approximately 2% carbon dioxide		
O ₂ per cent in gas	Vol. % O ₂ in blood	%CO ₂ in gas	%O ₂ in gas	Vol. % O ₂ in blood
3.	4.32	1.92	8.4	6.30
8.3	7.58	2.02	12.7	8.06
12.63	9.10	2.22	17.14	8.31
17.35	9.53	2.34	43.94	9.69
46.4	10.69	2.1	97.9	10.36
100.	11.66

TABLE XVII

Data show the oxygen and carbon dioxide contents of the blood of the black horse, *Cybleptus elongatus* (Le Sueur), when equilibrated with approximately 20% oxygen to which different percentages of carbon dioxide had been added. Data are arranged both chronologically and in order of increase in carbon dioxide.

Per cent CO ₂ in O ₂ equilibrated with	Vol. per cent CO ₂ in blood	Vol. per cent O ₂ in blood	Temperature °C
.03	8.44	16.10	24
.1	9.82	15.47	24
.22	10.50	14.76	24
.41	13.57	15.15	23
1.8	20.75	13.77	23
3.36	28.05	12.64	24

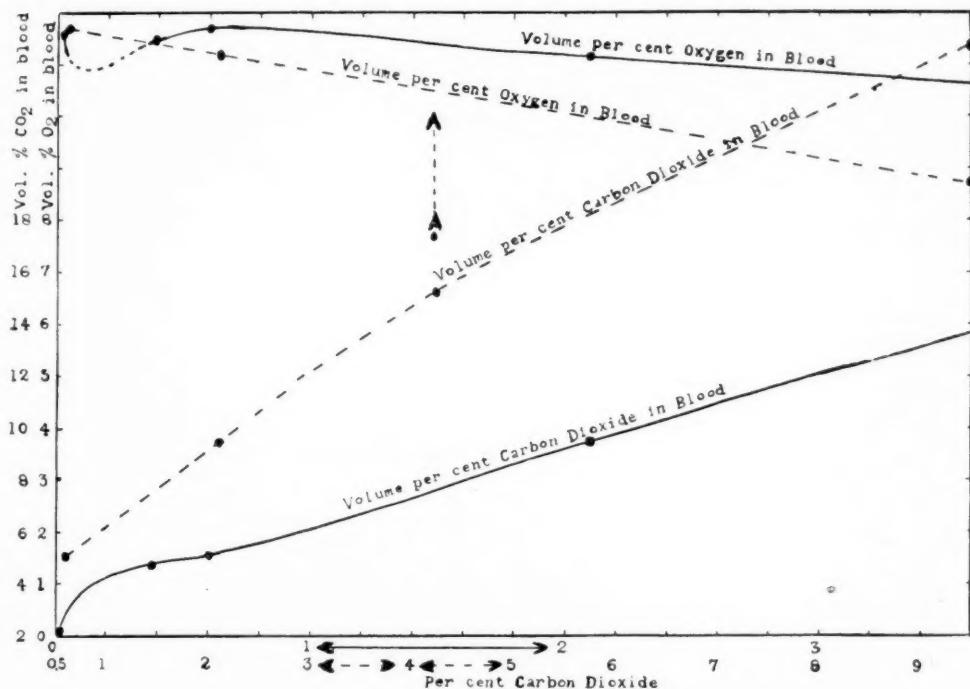


Figure 12 is a graphic representation of data given in Table XV.

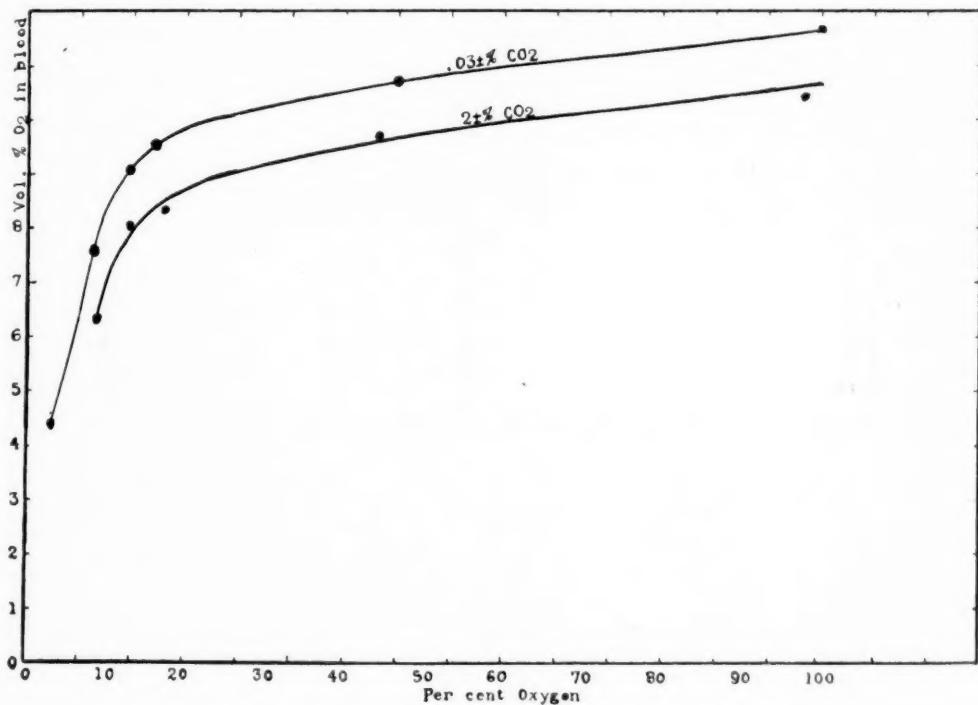


Figure 13 is a graphic representation of data given in Table XVI.

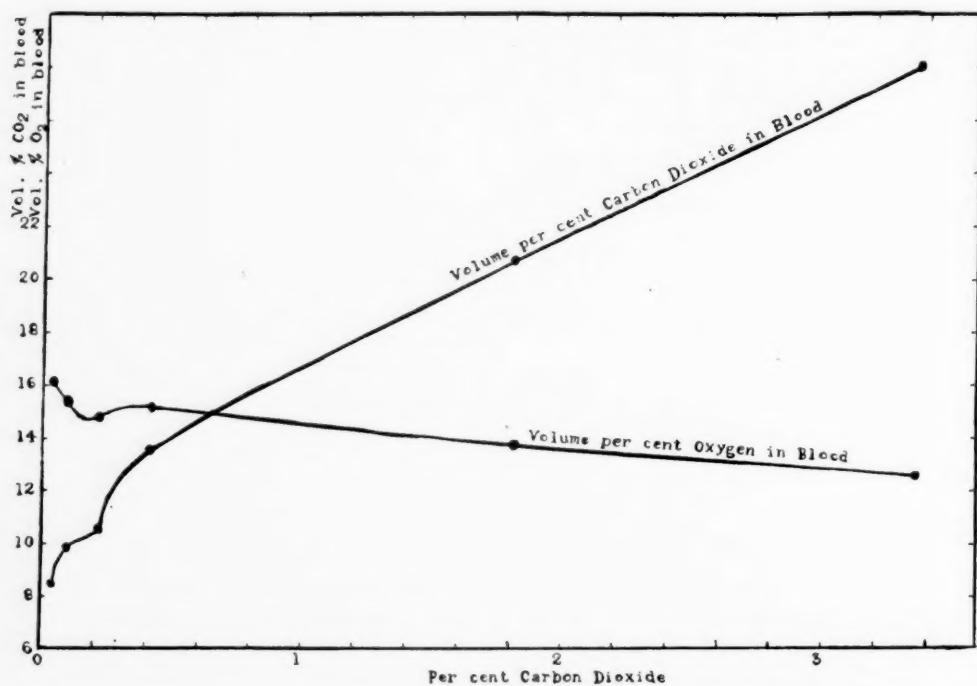


Figure 14 is a graphic representation of data given in Table XVII.

TABLE XVIII

Data show the oxygen and carbon dioxide contents of the blood of the channel or blue cat, *Ictalurus punctatus* (Rafinesque), when equilibrated with approximately 20% oxygen to which different percentages of carbon dioxide had been added. Data are arranged both chronologically and in order of increase in carbon dioxide.

Per cent of CO ₂ in O ₂ equilibrated with	Volume per cent of CO ₂ in blood	Volume per cent of O ₂ in blood	Temperature, °C
.037	.42	8.93	26
.22	1.82	8.51	26
.53	2.52	8.72	27
1.74	7.21	8.37	28
2.80	9.51	8.28	28
4.20	13.81	8.29	28
7.60	17.62	8.07	28

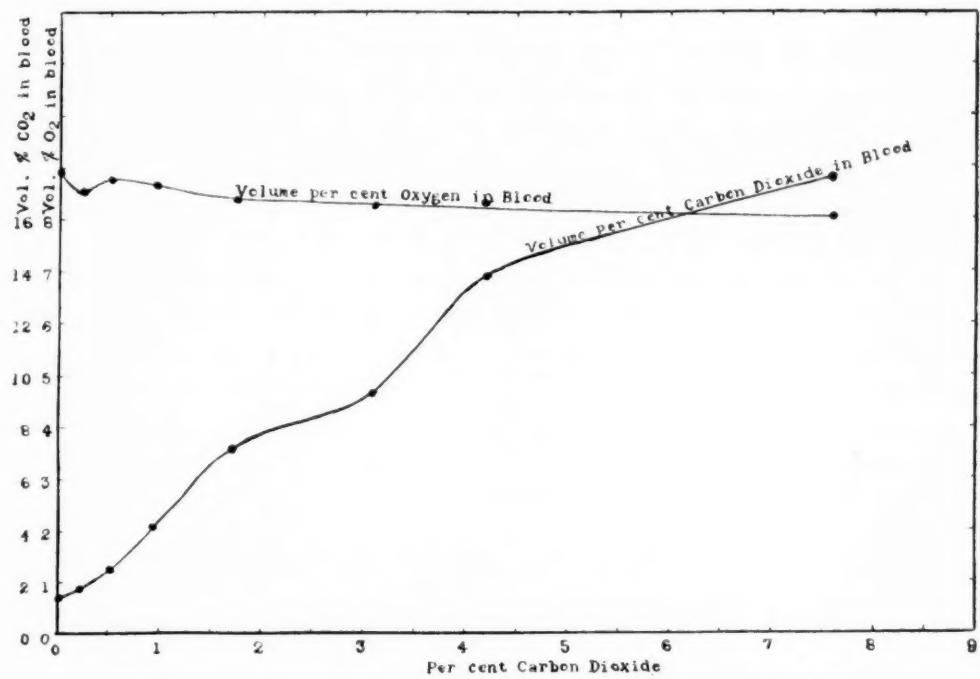


Figure 15 is a graphic representation of data given in Table XVIII.

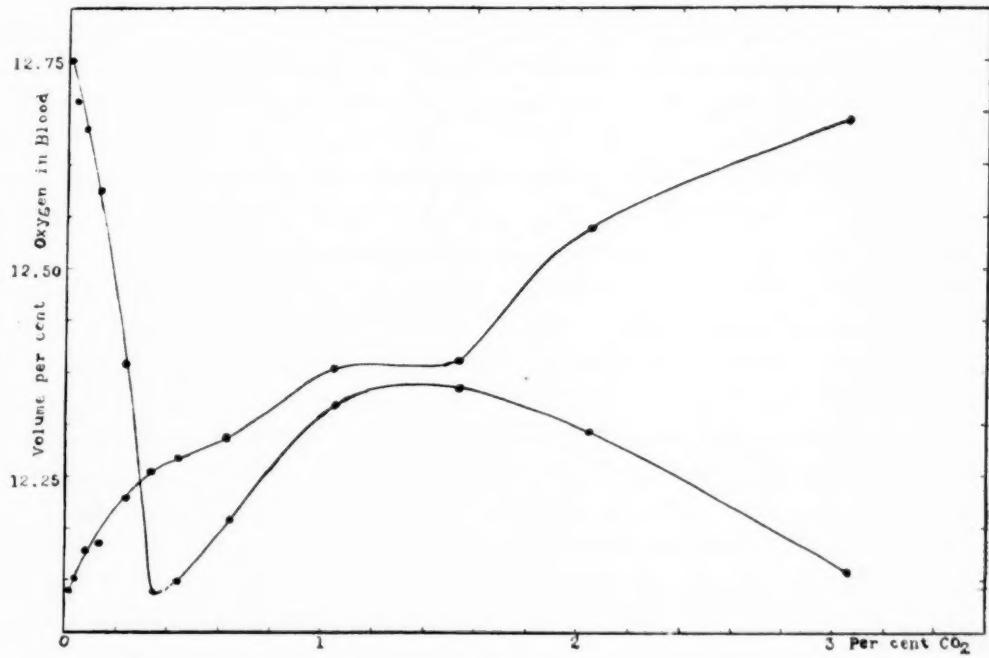


Figure 16 is a graphic representation of data given in Table XIX.

TABLE XIX

Data show the oxygen and carbon dioxide contents of the blood of the yellow cat, *Leptops olivaris* (Rafinesque), when equilibrated with approximately 20% oxygen to which different percentages of carbon dioxide had been added. Data are arranged both chronologically and in order of increase in carbon dioxide. 23° C.

Per cent of CO ₂ in gas mixture equilibrated with	Per cent of O ₂ in gas mixture equilibrated with	Volume per cent of CO ₂ in blood	Volume per cent of O ₂ in blood
.015	20.00	.82	12.75
.037	20.00	1.03	12.70
.078	20.00	1.53	12.67
.139	19.99	1.63	12.59
.239	19.97	2.52	12.39
.344	19.96	3.08	11.98
.444	19.96	3.35	11.99
.638	19.94	3.79	12.20
1.06	19.90	5.07	12.34
1.54	19.85	5.20	12.36
2.05	19.80	7.81	12.30
3.06	19.70	9.89	12.01

VII. RELATION OF THE PH TO THE CARBON DIOXIDE TENSION USED TO MODIFY THE BLOOD

BY EDWIN B. POWERS AND THRESSA A. HICKMAN

The gaseous tensions of natural waters in which fishes are found presumably approximate the partial pressures of these gases in the atmosphere. It has long been known that the so-called more sensitive fishes could not stand a sudden transfer to a different kind of water. This is seemingly true despite the fact that the same species of fish could be found normally living in the two waters.

Shelford and Allee (1913) state that the carbon dioxide in the water is an index to its suitability for fishes. Still there has been no agreement among biologists as to the responses of fishes to the different environmental factors. It is, thus, important to determine if possible the effect or effects, if any, of carbon dioxide tension upon the blood of fishes. This tension effect becomes of prime importance when it is considered that the blood supplying the gas gland of the swim-bladder is exposed to the pressures varying from almost nil in the surface and shallow water fishes to enormous pressures in deep sea fishes. Schloesing and Richard (1896) reported 85% oxygen and 12% nitrogen and argon. The remaining 3% carbon dioxide as calculated by Haldane (1898) is equivalent to approximately 5 atmospheres. If carbon dioxide partial pressure of the atmosphere be taken to be .033%, its partial pressure in the swim-bladder would be more than 15,000 times that in the atmosphere. Blood would have to be exceedingly stable to stand up under such carbon dioxide tensions and maintain its carbon dioxide and oxygen carrying capacities.

METHODS

A modified Cullen's (1929) micro-quinhydrone electrode equipped with a Leeds & Northrop potentiometer was used in the pH determinations. All blood was equilibrated in 30 ml tonometers at room temperature and barometric pressure. No corrections were made for barometric pressure. It was our purpose to determine indirectly whether or not the carbon dioxide carrying capacity of fish blood could be modified by being exposed to carbon dioxide tensions higher than that ordinarily met with by fishes in nature.

Blood was drawn directly from the heart by a Luer syringe in which had been placed a sufficient amount of potassium oxalate to prevent coagulation. In the last series of experiments (Table XXII) ammonium fluoride was also added. A sample of blood was then immediately equilibrated with air from a constant source and the pH readings taken. Another sample of blood was then equilibrated with a carbon dioxide partial pressure higher than that of air. This sample was again equilibrated with air and another set of pH readings taken. If the blood was equilibrated with a second or third carbon dioxide partial pressure a second and third sample of blood was taken. Control pH readings were always taken on a new sample of blood.

EXPERIMENTAL DATA

Samples of bloods of a number of different fishes were equilibrated at various carbon dioxide pressures greater than the carbon dioxide partial pressure of the air (Table XX and Figure 17) and pH readings taken again when equilibrated with air. It was found that without exception the pH readings were lower than when equilibrated with air, without first having been equilibrated with a higher carbon dioxide tension. To assure ourselves that it was not a progressive change taking place in drawn blood due to the formation of lactic acid from glucose (Evans, 1922) determinations were made on blood to which ammonium fluoride was added. This time (Tables XXI and XXII) controls were run. The time element was ruled out by always taking a control reading after the pH reading of a sample of blood previously equilibrated at a given higher carbon dioxide pressure. Again, the pH of blood previously treated at higher carbon dioxide tensions was without exception lower than the pH of the untreated blood. Instead of being lowered the pH readings of the untreated blood had a tendency to become progressively higher. There was some indication of this tendency in treated blood. This was attributed to large amounts of dry gases being used in the equilibrations. When moist gases were used this tendency did not appear.

In connection with some of our other work, it was noted that the handling of fish blood became more difficult if it happened to be contaminated with fluids from the body or injured tissues. A series of experiments were then run, in which a part of blood which had been drawn very carefully was con-

taminated with a small amount of fluid from the body and injured tissues. Both parts of the blood were then tested as before. See Table XXII and Figure 19. It was found that the untreated blood always had a higher pH than the treated blood. It was also noted that the treated blood on standing changed slightly more rapidly than did the untreated blood. Both bloods showed the same characteristic of being modified by higher carbon dioxide tension as was shown in all other bloods.

DISCUSSION

During the experimental work leading to the formulation of the first carbon dioxide dissociation curve ever drawn (Christainsen, Douglas and Haldane, 1914) it was found "that the blood in the saturator was undergoing progressive alteration" when "several determinations were made successively with the same sample of blood at varying pressures of CO₂." Christainsen, Douglas and Haldane, in describing this progressive alteration, state, "Thus the first determination of series with the same sample of blood was made at 40.0 mm CO₂ pressure, and gave 51.7 vols. of CO₂ per 100 cc of blood. After five hours, during which a series of determinations had been made at other pressures, a second determination was made at 40 mm. and gave only 41.2 vols." Evans (1922) suggested that glucose in the blood gradually changes to lactic acid. To prevent the change of glucose to lactic acid, ammonium fluoride is generally added, in addition to other substances used to prevent coagulation. The formation of lactic acid from glucose seems not to be a sufficient explanation as to why blood undergoes slow changes outside the body. The practice in exact work is to obtain a fresh supply of blood for each determination. Haldane, more than any other physiologist, has pointed out "the exactitude of normal physiological regulations." In speaking of these early experiments, he states (1922), "At the outset we wasted much time, however, through failing to realize that it was necessary to have the blood fresh for each experiment, as blood outside the body undergoes slow changes which diminish its capacity for carrying CO₂."

It was our purpose in this series of experiments to determine whether or not the gradual change taking place in the drawn fish blood could be accelerated by exposing the blood to carbon dioxide tensions higher than the carbon dioxide tensions of natural waters met with by fishes.

It is not surprising that we did find that fish blood, a very delicately balanced physico-chemical system, does undergo a marked physico-chemical change when exposed to carbon dioxide tension ten and a hundred and more times greater than the carbon dioxide tensions of the water normally bathing the gills of fishes. This change in the carbon dioxide tensions is sufficient in a stable physico-chemical system to raise the hydrogen ion concentration from ten to one hundred and more times.

No explanation is given as to the cause or the nature of the changes that take place in the drawn blood. But the fact that these changes can be accelerated by merely increasing the carbon dioxide tension of the blood is very important in the physiology of respiration of fishes.

TABLE XX

Shows the pH of the blood of different species of fishes when equilibrated with air after having been equilibrated with air to which different percentages of carbon dioxide had been added. The per cent of carbon dioxide with which the blood had been equilibrated is placed at the head of each column.

Common name of fish	.03% CO ₂ pH	0.6% CO ₂ pH	1.2% CO ₂ pH	2.12% CO ₂ pH	Temperature, °C
Black horse.....	7.66 7.65	7.52 7.52	7.47 7.47	7.44 7.44	28
Black horse.....	7.66 7.42	7.45	7.28 7.32	7.21 7.22	25
Sturgeon.....	6.90 6.90	6.82 6.82	6.79 6.79	6.72 6.79	25-26
	.03% CO ₂	.46% CO ₂	1.8% CO ₂	3.36% CO ₂	
Yellow cat.....	7.32 7.32	7.20 7.18	7.15 7.15	24
German carp.....	7.32 7.32	7.25 7.24	7.20 7.20	22
Drum.....	7.50 7.46	7.38 7.39	7.34 7.34	20
Black horse.....	7.67 7.67	7.50 7.50	7.46 7.46	7.41 7.38	26
Blue cat.....	7.39 7.39	7.37 7.36	7.35 7.35	7.27 7.25	21

TABLE XXI

A comparison of the pH of the blood of the black horse, *Cycloleptus elongatus* (Le Sueur), when equilibrated with air after having been equilibrated with air to which different percentages of carbon dioxide had been added and to which no carbon dioxide had been added. Data are arranged chronologically as well as in order of increase of carbon dioxide tension. Data of horizontal columns represent observations made as nearly simultaneously as possible. Neither sample of blood had come in contact with mercury. 25° C.

.03% CO ₂ pH	0.46% CO ₂ pH	1.8% CO ₂ pH	3.36% CO ₂ pH	100% CO ₂ pH
7.62 7.62	7.52 7.54	7.49 7.47	7.48 7.49	7.50 7.50
.03% CO ₂	.03% CO ₂	.03% CO ₂	.03% CO ₂	.03% CO ₂
7.62 7.62	7.65 7.65	7.65 7.66	7.67 7.65	7.67 7.67

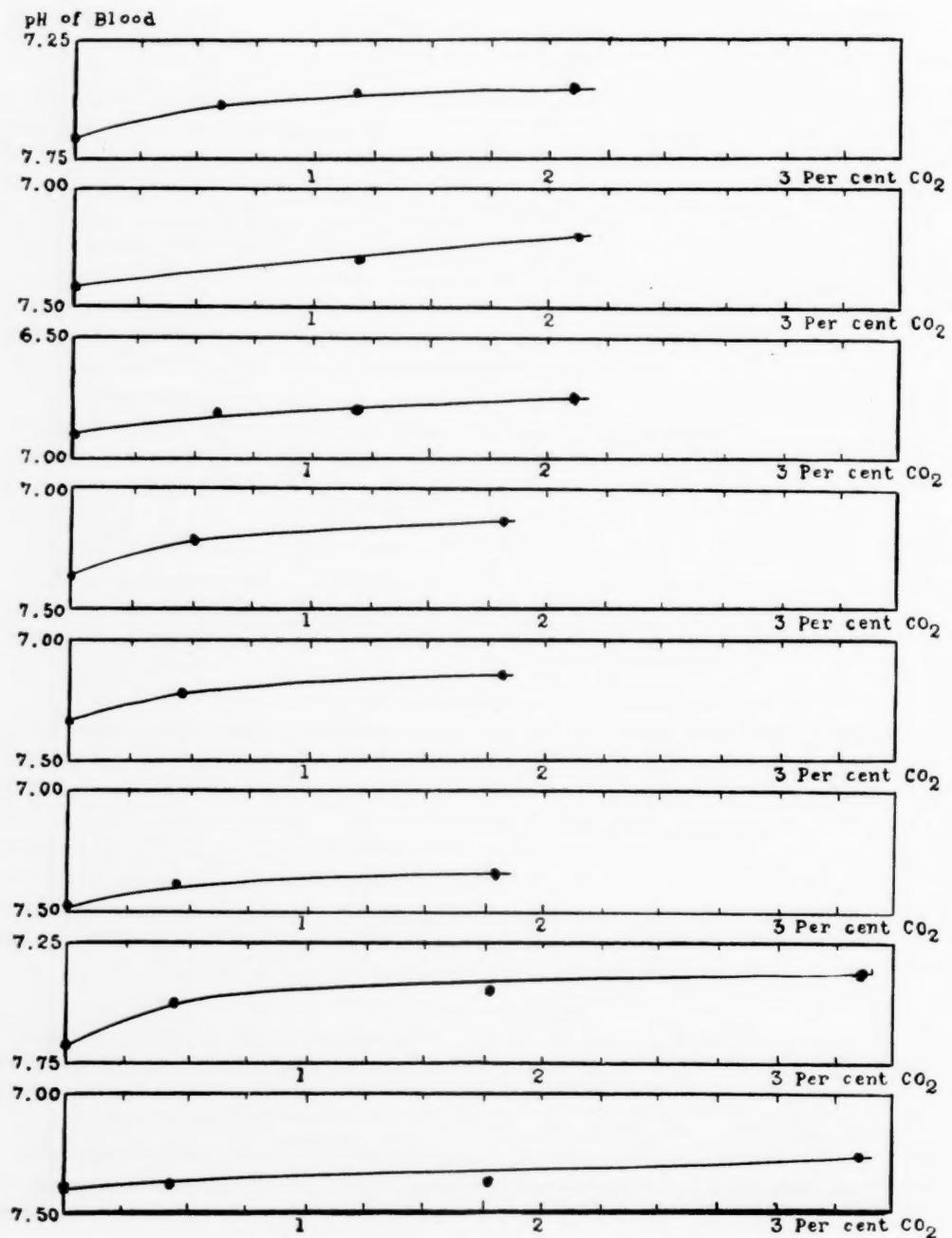


Figure 17 is a graphic representation of data given in Table XX.
The graphs are arranged in the same order as the data.

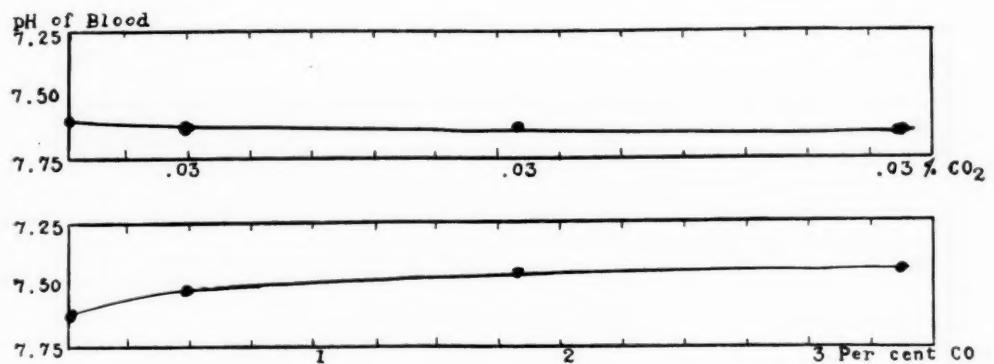


Figure 18 is a graphic representation of data given in Table XXI.

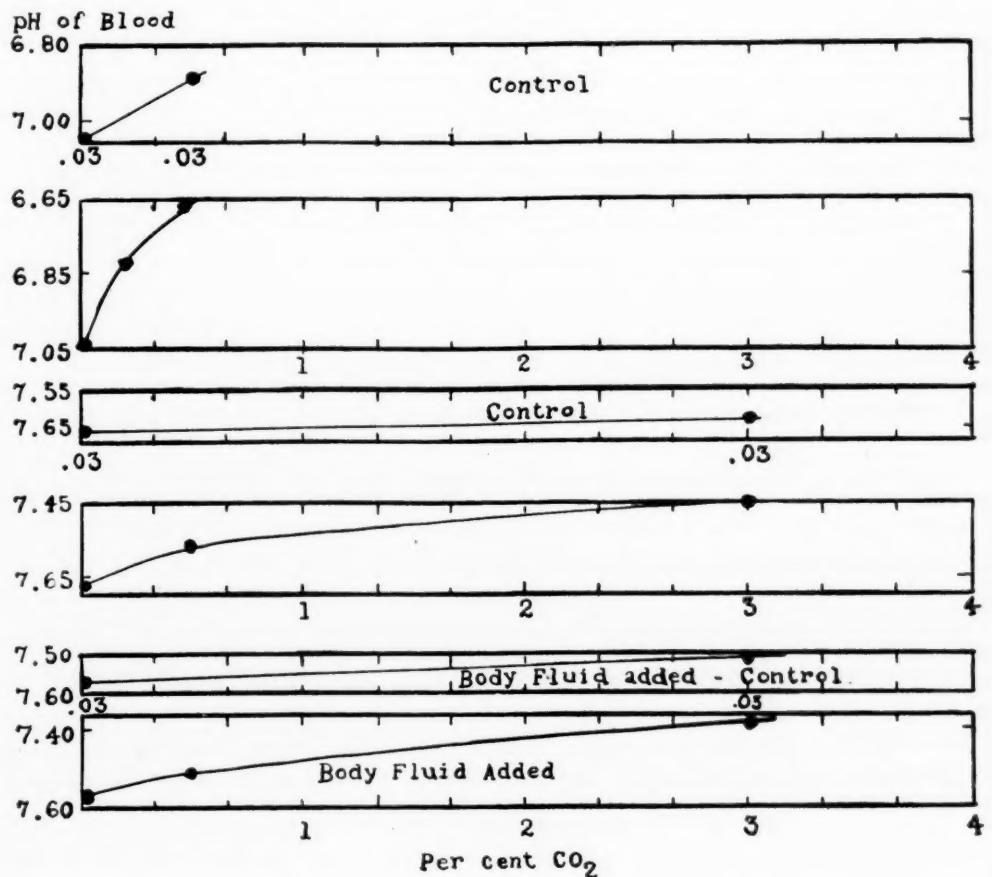


Figure 19 is a graphic representation of data given in Table XXII.
The graphs are arranged in the same order as the data.

TABLE XXII

Data are the same as given in Table XXI, except body fluid was added to one of the two samples of blood of the second fish. 25° C.

Red horse		Black horse—Blood not contaminated with body fluid				Black horse—Blood contaminated with body fluid		
pH .03% CO ₂	pH .03% CO ₂	pH .03%	pH .03%	pH .03%	pH .03%	.03%	.03%	
7.06	6.87	7.67	7.63	7.58	7.51
7.04	6.89	7.65	7.62	7.57
.03%	pH .2% CO ₂	.46%	.03%	.46%	3.0%	.03%	.46%	3.0%
7.06	6.85	6.65	7.67	7.56	7.46	7.58	7.50	7.39
7.04	6.82	6.65	7.65	7.56	7.45	8.57	7.50	7.39

VIII. NOTES ON THE FORMATION OF CRYSTALS IN DRAWN BLOOD

BY EDWIN B. POWERS, LULA MAE SHIPE, THRESSA A. HICKMAN

In this work on the physiology of respiration of fishes, each one of us was assigned to a special part of the problem. The duty of one of us, Miss Shipe, was to enumerate the red blood corpuscles of the blood of the fishes under various experimental conditions. The following of the behavior of the red blood corpuscles of drawn blood was also a part of this task.

During the progress of the enumeration of the red blood corpuscles of the German carp, *Cyprinus carpio* Linnaeus, which had been subjected to high carbon dioxide tensions, particles at first thought to be foreign substances were observed. Fresh perfectly clean Hayem's solution was prepared. Again particles other than blood corpuscles appeared. On closer examination and by aid of smears, it was determined that these particles were nuclei of broken down red blood corpuscles. Many of the corpuscles appeared distorted both in the counting chamber and on the smears. This was noted, and it was decided that the distortion was due to crenation.

The blood of the sturgeon, *Acipenser sturio* Linnaeus, that had been kept in cold storage over night, was examined. Numerous crystals, similar to Figure 20, were observed on the inside of the red blood corpuscles. This left no doubt that what we had previously supposed were crenated cells were really crystals formed on the inside of the cells.

Blood was taken direct from the heart of a black horse, *Cycloleptus elongatus* (Le Sueur), and when examined many red blood corpuscles contained crystals. See Figure 20. After this observation it was not uncommon to find crystals on the inside of corpuscles, either formed before the blood was

drawn or immediately after. Other forms of crystals, see Figures 20 to 26,² appeared in the blood of different fishes at various intervals after being drawn.

An attempt was next made to follow the order of appearance of the different types of crystals. Carp blood was drawn untreated, and in ten minutes rod shaped crystals, Figure 20, had appeared in many of the corpuscles. In forty minutes bunches of needle forms had appeared. By close examination these can be observed in Figure 21. The first cubes or plates, Figure 22, appeared in about two hours and replaced the blunt rod forms, Figure 21, that had appeared following the formation of the needles. By the end of the third hour long needles, Figures 25 and 26, had become more abundant than the plates, Figure 22.

Many short rod crystals, Figures 24 and 25, but mostly long rods, Figure 26, were found in the blood of a jack salmon, *Stizostedion vitreum* (Mitchill), that had been left in cold storage over night. No crystals could be recovered with chloroform extractions. Rhombic plates, Figure 22, appeared after evaporation in water that had been used to extract the blood. These extracted crystals were clear. When these crystals are formed in blood and remain in it for a long period, they turn darker yellowish as they age.

Many other observations were made which will be described in order.

A few crystals, form Figure 20, were found in the red blood corpuscles of a red horse, *Moxostoma papillosum* (Cope), immediately following the drawing of the blood.

Old blood of a carp showed masses of needle-like crystals, Figure 26.

Blood taken from a jack salmon that had been dead for about two hours showed all types of crystals.

Crystals, Figure 20, appeared in the blood of a jack salmon three minutes after being drawn when no chemicals were added.

Types of crystals like Figures 20 and 21 were found in the blood of a black horse thirty minutes after it was drawn.

A red horse's blood after being drawn for some time showed the crystal types of Figures 25 and 26.

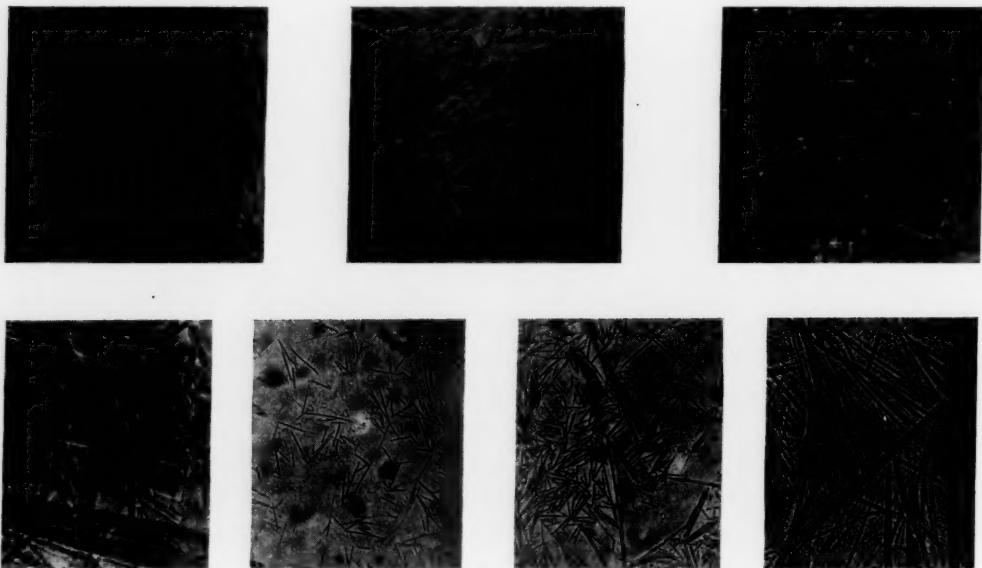
A black horse's blood showed types of Figures 20 and 21 when first drawn.

No attempt was made to determine the chemical nature of these various types of crystals. They were taken to indicate the breaking down or the modification of the hemoglobin, since crystals first appeared within the red blood corpuscles themselves and then passed through a more or less uniform cycle.

The observations were also taken to indicate that the hemoglobin of the fish blood is very easily broken down or at least modified through a more or

²We wish to thank Dr. C. D. Sherbakoff and Mr. J. O. Andes of the Experiment Station for making the microphotographs.

less uniform cycle by adverse conditions met with by the fish. Crystals were found in the blood taken directly from the blood stream of fishes which had been subjected to adverse conditions. These experiments are further described on pages 421-430, 430-436 and 438-442.



Figures 20 to 26 are microphotographs of crystals formed in drawn fish blood. The crystals in Figure 20 are within the red blood corpuscles. All Figures are of the same magnification. The granules are from nuclei of red blood corpuscles.

IX. THE EFFECT OF THE CARBON DIOXIDE TENSION WITH WHICH THE BLOOD HAS BEEN EQUILIBRATED UPON THE OXYGEN AND CARBON DIOXIDE CAPACITIES OF THE BLOOD*

BY EDWIN B. POWERS AND THRESSA A. HICKMAN

During the course of our work, it was found that when whole blood was equilibrated with gas mixtures containing carbon dioxide percentages greater than in air, the blood loses capacity to carry carbon dioxide and oxygen (p. 421). This loss in capacity to carry carbon dioxide and oxygen is in addition to the loss in capacity to load due to the carbon dioxide effect. Root (1931) has found this same carbon dioxide effect on oxygen carrying capacity in the blood of marine fishes.

The questions immediately arise: is this modification of the blood at higher carbon dioxide tensions irreversible; or will the blood on being again equilibrated with air at low carbon dioxide pressure regain its original capac-

* These observations are being published at this time because of their importance regarding the finding of denaturation, acid effects and inactivation of hemocyanin and hemoglobin by exposures to acids or high carbon dioxide pressures.

ity to unite with carbon dioxide and oxygen? This is of importance in the physiology of the gas transportation to and from the tissues and the preservation of the normal efficiency of the blood to transport carbon dioxide and oxygen. It also has its bearing on the mechanism of the deposition of gases into the swim-bladder of fishes.

MATERIALS AND METHODS

To test the reversibility of the blood, from 3 to 5 pound black horses, *Cyclopterus elongatus* (Le Sueur), and the Jack salmon, *Stizostedion vitreum* (Mitchell), were obtained. Blood was drawn direct from the conus arteriosus by means of a Luer syringe. Potassium oxylate and ammonium fluoride were used as anticoagulants. Only enough blood could be obtained from a fish to run a complete series of determinations when 0.2 ml samples were used.

The procedure was always first to determine the volume per cents of carbon dioxide and oxygen with the Van Slyke Gas Blood Apparatus after being equilibrated in a tonometer at constant temperature with air of a uniform composition. A second sample was equilibrated with air plus from 0.6 to 4.6% carbon dioxide. The sample was then brought into equilibrium with air and the volume per cents of carbon dioxide and oxygen determined. A third sample of blood was taken, equilibrated with air only and the carbon dioxide and oxygen contents determined. A fourth sample was equilibrated with pure carbon dioxide, then equilibrated with air, and the carbon dioxide and oxygen contents determined. This order was adhered to throughout the series of experiments, except when due to the small amount of blood obtained, a fourth sample was not run. Duplicates were run with only two exceptions.

In equilibration a small amount of blood was rotated in a tonometer for 15 to 20 minutes. The air in the tonometer was changed 3 to 4 times. All air passing into and through the tonometer was saturated with water vapor to prevent evaporation of the blood plasma. The 15 to 20 minutes rotation of the tonometer and 3 to 4 times of changing of air were sufficient to bring the blood back into equilibrium with air after having been equilibrated at higher carbon dioxide pressure, as is evidenced by the fact that the carbon dioxide volume per cent was lower with very few exceptions than when the blood was equilibrated with air only.

EXPERIMENTAL DATA

Table XXIII gives the results of these determinations. In the second, third and fourth columns are given the two sets of determinations made on blood that had been equilibrated with air only. The first set for each series of determinations are data obtained immediately after the blood was drawn. The data of the second set are determinations on a third sample. The second

sample of blood had always been equilibrated with air plus a carbon dioxide partial pressure greater than that of atmospheric air. The determinations in the pure carbon dioxide experiments were always made last. This was to allow 40 to 60 minutes to bring the blood back into equilibrium with air after it had been equilibrated with the pure carbon dioxide.

When the data are examined, it is found that with only three exceptions, the volume per cent of carbon dioxide of the blood samples that were first equilibrated with 3.5% carbon dioxide was always less than in blood equilibrated with air only. There are no exceptions in the pure carbon dioxide, the 0.6% and the 4.6% carbon dioxide experiments. There are more exceptions in the reduction of the volume per cent oxygen in the blood equilibrated with the air after having first been equilibrated with the higher carbon dioxide pressures, there being six exceptions in the 3.5% carbon dioxide experiments and two in the pure carbon dioxide experiments. When the volume per cent of the total gas, that is, carbon dioxide plus oxygen, is considered, it is found that there are only four exceptions to the rule that total gas volume per cent is less in blood that had been equilibrated with higher carbon dioxide pressures before being equilibrated with air than in blood equilibrated with air only.

When the volume per cents of carbon dioxide, oxygen and total gas are analyzed, it is found that with four exceptions the carbon dioxide, with eight exceptions the oxygen, and with six exceptions the total gas were lower in the second sample of blood equilibrated with air than in the first sample.

The results of these experiments, our previous findings (p 421) and the findings of Root (1931) indicate that when fish blood is brought to a higher carbon dioxide tension (0.6% of an atmosphere to approximately one atmosphere) higher than the carbon dioxide partial pressure of the atmosphere, the blood loses in capacity to combine with carbon dioxide and oxygen, which when outside the body, is not completely reversible. Anson and Mirsky (1925) claim that haemoglobin is completely denatured in about 3 minutes at 0° C with N/20 HC1 and that the denaturation is reversed to about 75% by neutralizing the acid with an equal amount of NaOH plus a small amount of cyanide. However, Cole and Boor (1930) suggest that Anson and Mirsky were not dealing with a true denaturation but with an even more obscure reaction peculiar to hemoglobin. Redfield and Mason (1928) have shown a similar acid effect on purified hemocyanin of *Limulus polyphemus*. Root (1931) calls it an inactivation.

In considering the extent of reversibility of the loss in capacity of blood to combine with carbon dioxide and oxygen on acid or carbon dioxide treatment, it must be remembered that drawn blood is undergoing a more or less rapid disintegration. This is especially true of the fish blood with which we have had experience. Our experimentations on fish blood have led us to be-

lieve that the stability or nonstability of the blood, at least to a certain extent, reflects the physiological condition of the fish when the blood is taken. Evidence that drawn fish blood is rapidly modified is furnished by the short time before crystals appear within the red corpuscles themselves. Also due to the fact that 0.2 ml had to be used and the carbon dioxide and oxygen capacities of the blood are low, the experimental error is relatively higher. Thus differences in certain of the determinations may be more apparent than real. However, there are on an average always a lowering of the capacities of the blood to combine with carbon dioxide and oxygen. From Table XXV, it is seen that there is very little permanent loss in the capacity of blood after exposure to higher carbon dioxide pressures to combine with oxygen. There is somewhat greater loss in the capacity to combine with carbon dioxide. This reduction of the active alkalinity is perhaps brought about either through modification of the protein molecules within the red corpuscles or by the precipitation of carbonates of alkaline earths, thus robbing the bicarbonates of the alkalies, or by otherwise modifying the blood.

With a lowered active alkalinity the blood would now be more acid at the carbon dioxide tension when in equilibrium with air. This is evidenced by the fact that the pH reading of the blood is always lower after having been treated with carbon dioxide at higher pressures (p. 430). This increased acidity would reduce the loading of the hemoglobin to oxyhemoglobin. This factor alone would perhaps account for the lower volume per cent oxygen of the blood first exposed to the higher carbon dioxide pressures before equilibrating with air. If the carbon dioxide acid combines with the protein as suggested by Redfield and Mason (1928) or is inactivated as suggested by Root (1931), these reactions, the acid or carbon dioxide effect upon the oxygen capacity of blood, are reversible even outside the fish. The loss in the capacity to unite with carbon dioxide seems to be more permanent. It is suggested that this might be due to the formation of very slightly soluble carbonates which are removed very slowly. Within the fish, with the large amount of blood in the body, the removal of the carbonates would perhaps be complete over a short or longer period of time.

TABLE XXIII

The Carbon Dioxide after-effect upon the Carbon Dioxide and Oxygen Capacities of Whole Blood. Data represent volume per cent gas content of blood when equilibrated with air at room temperature and atmospheric barometric pressure. *BH*, black horse; *WS*, white salmon.

Fish	Equilibrated with Air only First and second samples			Volume per cent of Carbon dioxide equilibrated with before equilibration with air						Temp- erature, °C	
				3.5% CO ₂			Pure CO ₂				
	CO ₂	O ₂	Total Gas	CO ₂	O ₂	Total Gas	CO ₂	O ₂	Total Gas		
BH.....	1.24	12.17	6.70	.64	12.48	6.06	.95	11.93	6.17	26	
	.98	12.60	6.79								
BH.....	1.30	10.81	6.06	1.35	9.69	6.02	.81	10.48	5.65	23-24	
	1.36	10.71	6.03								
BH.....	1.44	4.87	3.15	1.37	5.24	3.30	1.00	4.57	2.83	26	
	1.44	4.93	3.18								
BH.....	1.61	10.91	6.25	1.58	10.34	5.96	1.32	11.05	6.18	24	
	1.55	11.30	6.42								
BH.....	1.66	7.72	4.69	.57	9.07	4.82	25	
	1.63	7.72	4.67								
BH.....	1.38	8.26	4.82	1.68	8.50	5.09	.86	8.79	4.82	26	
	2.22	9.25	5.73								
BH.....	1.78	11.35	6.56	1.42	11.96	6.64	.98	11.27	6.12	25	
	1.77	11.18	6.48								
BH.....	1.90	7.05	4.47	1.47	5.59	3.53	1.11	8.59	4.85	26	
	1.81	7.94	4.87								
BH.....	2.22	11.10	6.66	1.21	10.17	5.69	1.27	10.08	5.67	26	
	2.23	10.53	6.38								
BH.....	2.64	8.67	5.66	2.28	8.63	5.45	26	
BH.....	5.47	3.16	4.31	4.77	3.70	4.23	22	
	5.05	3.17	4.11								
BH.....	8.81	13.74	11.28	4.04	13.52	8.78	4.76	12.74	8.75	26	
	7.85	14.58	11.22								
WS.....	2.25	7.43	4.84	2.21	7.31	4.76	1.83	6.27	4.05	24	
	2.32	8.19	5.20								
WS.....	3.58	3.18	3.38	2.67	2.99	2.83	24	
					6% CO ₂						
BH.....	5.35	13.03	9.19	3.42	12.47	7.94	19	
	4.70	12.50	8.60								
BH.....	5.35	12.53	8.94	3.37	12.31	7.84	19	
	4.71	12.71	8.72								
BH.....	4.88	13.47	9.18	4.23	9.75	23	
	4.82	13.25	9.04								

TABLE XXIV

The Table shows the per cent loss in the capacities of the blood to hold carbon dioxide and oxygen after having been exposed to carbon dioxide pressures higher than that found in air.

Sample of blood equilibrated with air only compared	Equilibrated with 3.5% CO ₂ before being equilibrated with air. % loss in capacity of blood	Equilibrated with pure CO ₂ before being equilibrated with air. % loss in capacity of blood
1st.....CO ² =	28	38
2nd....." =	24	35
1st.....O ² =	4	2
2nd....." =	5	4
1st.....total gas =	10	10
2nd.....total gas =	11	11

X. MECHANISM OF THE DEPOSITION OF GASES INTO THE SWIM-BLADDER

BY EDWIN B. POWERS

Since the time of Needham (1667), who it seems was the first to suggest a hydrostatic function for the swim-bladder, there have been various functions postulated and various suggestions made to explain the deposition of the gases into the swim-bladder. According to Bridge (1890), this hydrostatic theory was further elaborated by Borelli (1680). As early as the time of Priestley, Fourcroy, Configliachi, and Biot, who according to Quekett (1844) analyzed the gas contents, it has been known that the proportion of gases in the swim-bladder were not the same as found in the air. As Quekett states, "All agree in these points, that in those fishes with closed air-bladders, a great proportion of it (*gas*) consists of oxygen as much as from 69 to 87 per cent, whilst in those fishes in which there is a ductus pneumaticus as in the carp, nitrogen abounds, to as much as 87 per cent, whilst the oxygen and carbonic acid were only about 7 and 5 per cent respectively." This, though stated almost a hundred years ago (1844), expresses the extent of our knowledge at the present time. Delaroche (1809) held that the swim-bladder musculature was in a state of tonic contraction and the fish would sink or rise according to the contraction or relaxation of the musculature, and was perhaps the first to point out the exchange of gases between swim-bladder and blood. Biot (1807) and subsequent workers have determined that by emptying the swim-bladder of a part of its gas content either by reduction of atmospheric pressure or artificially by puncture, oxygen deposition is stimulated. Biot (1807) and Provençal and Humbolt (1809) pointed out the greater the depth at which the fishes are taken the greater the oxygen content. Oxygen deposition is stimulated (Haldane, 1898) by lowering a fish in a cage into deep water. The process can be reversed by bringing a fish from a greater depth to a lesser depth [Moreau, 1877 (Haldane, 1898) and subsequent workers]. There is also an immediate decrease in volume of the swim-bladder with an immediate increase in depth, and *vice versa*.

Aside from a hydrostatic function of the swim-bladder other accessory functions are suggested as (Quekett, 1844) purification of the blood and the gas contained in the swim-bladder, respiration (Poey, 1856-1858, 1858, Wilder, 1875, Moreau, 1876, Bridge, 1890, Mark, 1876, Potter, 1927, and Toryu, 1927) and sound production (*in certain fishes*) (Sorensen, 1895 and Greene, 1924, 1924b).

Since the time of Biot (1807) and Delaroche (1809) to the present time it has been considered that there is an active secretion of gas. Moreau (1876) found that deposition ceased when the sympathetic nerve was sectioned. This was further investigated by Bohr (1892, 1894). Bohr performed experiments

that show without doubt that by lowering the mechanical pressure on the fish, gas was absorbed from the swim-bladder, and by increasing the mechanical pressure, gas was "secreted," and also that there was an increase in oxygen percentages of the gas content of the swim-bladder after puncture, and states, "I have never known this experiment to fail. . . . Action is not very marked in less than 6 hours . . . while in all examined cases 24 hours were enough." He also performed experiments which showed conclusively that the gas deposited was not pure oxygen "but a mixture containing about 80% oxygen." He further states that "secretion" ceased after the sectioning of the vagus. "Secretion" was intensified when the rami intestinales or the two rami cardiaci or the nervi laterales were cut. He thus concludes "that the formation of gas in the air-bladder is a true secretion of a highly oxygenated gaseous mixture; and that the secretion is so far under the control of the nervous system that it fails when the branches of the vagus which supply the air-bladder are cut. . . ." Since Moreau had found a slight increase in oxygen percentages after the sectioning of the sympathetic, Bohr further concluded that "The vagus would control the filling, and the sympathetic the emptying of the air-bladder." Schloesing and Richard (1896) supposed that the oxygen had accumulated in the swim-bladder during the ascent from deeper waters by oxygen arising from the decomposition of hemoglobin due to decreased pressure. In 1898 Haldane set forth again the notion that the mechanism was pure secretion and that the secreted gas was a mixture composed mostly of oxygen. Jaeger (1904 and 1906) and Woodland (1908) maintain that a substance secreted by the gas-gland itself brings about the "secretion" of oxygen.

All workers have found that the power of a more or less rapid deposition of gas is always accompanied by the presence of a so-called gas gland which is always associated with a rete mirabile. This rete mirabile, *i.e.*, this wonderful net, was first described by Quekett (1844) although the name dates back to Redi (1684). However, it was Woodland (1911) who worked out the details or rather worked out the fundamental mechanics of the rete mirabile-gas gland relations. These can be described as parallel arteries and veins which are split up into a large number of capillaries, the rete mirabile, in which the capillaries of the arteries run parallel to the capillaries of the veins. The capillaries of the veins and those of the arteries always approximate each other and are, as Krogh (1929) states, "intercalated with the most astonishing regularity." A venous capillary is always surrounded by a number of arterial capillaries. According to Krogh these capillaries are about eight times as long as muscle capillaries which are otherwise among the longest in the body. At the end of the rete mirabile proximal to the gas gland the arterial and venous capillaries again form into arterioles and venules and arteries and veins which are united by arterioles to epithelial capillaries in the

gas gland to venules and back to capillaries of the rete mirabile. In some forms the capillaries of the rete mirabile do not unite to form arterioles and venules but are continuous with the glandular epithelial capillaries. However, the mechanics are the same in all cases.

This brings us up to the present state of knowledge regarding the deposition of gases into the swim-bladder of fishes and the morphology of the rete mirabile and gas gland. It now becomes necessary to consider the blood of vertebrates in general and of fishes in particular as a physiological-physico-chemical system, since it is obvious that when gas is deposited into the swim-bladder it is extracted from the blood that supplies the rete mirabile and the gas gland.

The form of the oxygen dissociation curve (Bohr, 1904) and the influence of the partial pressure of carbon dioxide on its form (Bohr, Hasselbalch and Krogh, 1904) and the form of the carbon dioxide dissociation curve (Christiansen, Douglas and Haldane, 1914) of the blood of mammals are too well known to warrant descriptions here. In our own laboratory, experiments have been performed on fish blood to obtain information regarding the blood of fishes as a physiological-physico-chemical system. These experiments have already been described in preceding pages. It is necessary here to give only a brief summary of the results of these experiments. It was found (see p. 430) that if fish blood is equilibrated with a higher carbon dioxide pressure than that ordinarily met with by the fish, the blood is modified, becoming more acid. That is, the pH of the blood equilibrated with atmospheric air, after having been equilibrated with air plus a higher carbon dioxide partial pressure than that of air, is lower than the pH of the same blood that had not been so treated but had been equilibrated with atmospheric air only. No exceptions were found. To assure ourselves that this was not due entirely (Christiansen, Douglas and Haldane, 1914) to the blood in the saturator undergoing a progressive alteration due to the formation of lactic acid from glucose (Evans 1922) but primarily to the effect of the higher carbon dioxide pressure, we took two samples of the same blood; one was equilibrated with different carbon dioxide partial pressures and the other only with the carbon dioxide partial pressure of the atmosphere, and found that the treated blood when again brought into equilibrium with the atmospheric air always had a lower pH than the untreated blood. Thus this alteration of the blood could not have been due entirely to a time element. As a matter of fact the pH of the untreated blood was always determined following the pH determinations of the treated blood. From this it would follow that fish blood after having been treated with a carbon dioxide partial pressure even as small as .46% of an atmosphere at laboratory temperature and pressures loses a part of its carbon dioxide carrying capacity.

To assure ourselves from another angle that this is true, we took a sample

of blood and measured its carbon dioxide capacity when equilibrated with air. Its carbon dioxide capacity was found to be 5.39 volume per cent. We then divided the blood into two approximately equal portions. One portion was brought into equilibrium with air plus .46% carbon dioxide and then again equilibrated with air and the carbon dioxide capacity again determined. Its carbon dioxide capacity was found to be 3.41 volume per cent while the carbon dioxide capacity of the untreated blood (determined after the determinations were run on the treated blood) was 4.69 volume per cent. The first tests were made with blood that had been treated only with potassium oxalate. The observations were then repeated with another sample of blood to which both potassium oxalate and ammonium fluoride had been added. The carbon dioxide and oxygen capacities were found to be 5.35 and 13.03 volume per cent immediately after being drawn and equilibrated with air and 3.42 and 12.47 when equilibrated with air after having been equilibrated with air plus .6% CO₂, while a portion of the same sample of the blood which had been equilibrated with air only had carbon dioxide and oxygen capacities of 4.70 and 12.50 volume per cent. In this last portion of blood the Van Slyke determination was run after the first two had already been run. Tests were again made with the results shown in Table XXV.

TABLE XXV

Data show the effect of carbon dioxide on the subsequent carbon dioxide and oxygen capacities of the whole blood of the black horse, *Cyclopterus elongatus* (Le Sueur). All determinations were made after blood was equilibrated with air. The blood samples were from different fishes.

Blood when first drawn		Blood treated with CO ₂		Untreated blood	
CO ₂ vol. %	O ₂ vol. %	CO ₂ vol. %	O ₂ vol. %	CO ₂ vol. %	O ₂ vol. %
0.6% CO ₂					
5.35	12.83	3.37	12.31	4.71	12.71
4.6% CO ₂					
4.85	13.47	4.23	9.75	4.82	13.25

All subsequent experiments (p. 438) showed the same falling off of the carbon dioxide and oxygen capacities of whole blood after being treated with carbon dioxide at a pressure higher than the tension ordinarily found in natural waters.

This leaves no doubt that, at least in fish blood, a part of the carbon dioxide carrying capacity is lost after being treated with carbon dioxide at a pressure higher than that of the carbon dioxide partial pressure of the atmosphere, *i.e.*, higher than the carbon dioxide tensions to which fishes are subjected under normal conditions.

Wastl (1928) also found that carp blood is modified when treated with higher carbon dioxide pressures than normal to the fish. Haggard and Henderson (1920, 1920a) found that both defibrinated and oxalated mammalian blood are modified in the same way when exposed to carbon dioxide pressures lower than normal.

The oxygen dissociation curves of the blood of the yellow cat, *Leptops ovularis* (Rafinesque), at .03% carbon dioxide and at approximately 2% carbon dioxide were then compared (see p. 421). It was found that the two curves did not have the same relation that is always noted when the same sample of mammalian blood is equilibrated at different carbon dioxide tensions but their relations are as two distinct bloods would be, one having a higher oxygen capacity than the other. That is, the oxygen capacity of the blood of fishes is modified, its oxygen capacity being lowered, by a carbon dioxide pressure of 2% which is higher than that of the carbon dioxide partial pressure of the atmosphere. These two curves compare favorably with Figure 33 (better illustrated by Figure 34) of Barcroft (1925) which are oxygen dissociation curves of blood modified by equal volumes of serum to which unequal amounts of red-blood corpuscles had been added. The two curves of Figure 13, p. 427, show that the oxygen capacity of the blood had actually been reduced when equilibrated with 2% carbon dioxide. That is, there was an actual loss of capacity to carry oxygen, aside from the unloading of oxygen by the oxyhemoglobin to form hemoglobin at the higher carbon dioxide tension.

Just as the preparation of this paper had been completed for publication a paper by Root (1931) has appeared in which it is shown that the loss in the capacity of the blood equilibrated at 25 mm Hg carbon dioxide to combine with oxygen in the sea robin, *Prionotus carolinus* (Linneaus), approximates 75 per cent. Regardless of whether this is due to the inactivation of the hemoglobin, denaturation, or other changes taking place in the hemoglobin the fact remains that fish blood loses in capacity to hold oxygen at higher carbon dioxide tensions. This decrease in the capacity of fish blood to hold carbon dioxide and oxygen in addition to what Root calls the Bohr effect are essential factors, as will be shown in the following pages, in the deposition of gases into the swim-bladder. Our experiments (p. 438) indicate that the blood on being brought back to lower carbon dioxide tensions regains at least, in greater part, its original oxygen carrying capacity.

We will now sum up known facts in an attempt to formulate an explanation of the mechanism of deposition of gases into the swim-bladder of the fish. From data here presented (Tables XXVI and XXVII) on the gas contents of the swim-bladders of two species of fishes, the blue cat, *Ictalurus punctatus* (Rafinesque), and the carp, *Cyprinus carpio*, Linnaeus, and on the gas contents of the swim-bladders mostly of fishes having no ductus pneu-

matus, taken from literature cited, it seems that the gas content or gaseous partial pressures of the water has little or no effect upon the actual gaseous complex of the swim-bladder. The gaseous complex seems to be determined more by other factors, such as external pressure. However, Pearse and Achtenberg (1920), Greene (1924), Hall (1924), Potter (1927) and others have reported the effect of the amounts and kinds of dissolved gases. The data of my own observations and those cited indicate that gaseous deposition into the swim-bladder is not primarily by the process of simple diffusion due to the tension of the oxygen in the arterial blood as it is carried to the rete mirabile. This is the conclusion of most if not all modern workers on the

TABLE XXVI

The data are arranged in the order of increase in the carbon dioxide tension of the water, column two. Column three gives the oxygen content of the water. Column four gives the per cent of carbon dioxide and five the per cent of oxygen in the swim-bladder of the Blue Cat, *Ictalurus punctatus* (Rafinesque), after having been kept in the experimental water for from four to seven hours.

Exp. No.	CO ₂ of H ₂ O in mm of Hg	O ₂ of H ₂ O in cc per l	Volume % CO ₂ in swim-bladder	Volume % O ₂ in swim-bladder
39	1.63	3.07	.22	16.55
40	1.92	3.60	.75	15.17
21	2.19	10.20	.05	17.54
22	2.19	10.42	+	19.40
7	2.30	2.65	.21	19.48
16	2.31	.35	.35	18.44
18	2.47	1.22	.12	19.45
10	2.61	1.23	.41	18.85
19a	2.84	.63	.55	15.69
20	2.88	2.12	.02	18.36
11	2.90	1.91	8.89	12.65
41	2.91	4.23	+	15.91
12	2.93	2.35	.17	17.53
13	2.93	1.97	.22	19.48
15	2.94	.90	.70	18.52
14	3.02	.96	.17	19.11
6	3.15	2.01	.31	19.80
27	3.15	15.83	.23	16.79
33	3.26	13.27	.50	18.54
34	3.59	14.42	.20	20.16
29	3.62	14.58	.18	13.41
30	3.70	12.83	.31	17.39
8a	3.74	2.02	8.22	15.16
8b	3.74	2.02	1.91	15.94
28	3.78	15.46	.48	11.18
32	3.81	19.96	.24	18.27
31	3.86	22.65	.41	17.99
9	4.15	.92	.26	17.66
42	4.18	4.76	.10	17.90
36	4.39	.96	.22	14.66
35a	4.53	1.39	.33	18.48
35b	4.53	1.39	.42	20.09
37	4.92	1.00	.44	17.97
25	5.94	1.91	3.01	12.19
23	8.57	5.97	.21	18.23
38	11.08	.65	.61	18.46
19b	2.84	.63	+	18.21

TABLE XXVII

The Table gives the same data and arrangement as Table XXVI. But in this case the German Carp (*Cyprinus carpio*, Linnaeus) was used.

Exp. No.	CO ₂ of H ₂ O in mm of Hg	O ₂ of H ₂ O in cc per l	Volume % CO ₂ in swim-bladder	Volume % O ₂ in swim bladder
47	2.70	6.33	2.14	4.34
48	3.29	6.14	2.60	4.59
52	3.75	6.18	1.47	5.93
51	3.75	6.03	1.08	6.17
43	3.76	4.76	1.65	6.95
44	3.76	4.76	3.90	11.59
50	3.77	5.99	.49	10.51
46	3.79	5.68	6.26	4.49
49a	3.80	5.82	1.37	7.97
49b	3.80	5.82	2.48	10.07
57	4.30	26.48	2.24	6.46
55	4.44	22.08	4.31	7.95
53	4.60	10.28	1.31	7.32
56	4.63	21.09	2.38	17.00
58	4.63	17.52	2.49	8.28
59	4.93	3.01	3.66	13.99

subject. In the two sets of experiments, one on the blue cat and the other on the carp, it is found that in each case the carbon dioxide contents of the swim-bladder were higher on an average at higher carbon dioxide tensions of the water. See Tables XXVI and XXVII. The oxygen contents showed no such relations. It is possible that the experiments were not of sufficiently long duration, from $2\frac{1}{2}$ to 7 hours, to reflect the gaseous complex of the water. Bohr (1894) pointed out that very little change takes place in the gas complex of the swim-bladder during the first six hours. My own observations and the experiments of Moreau (1876) and Bohr (1894) suggest a nerve control of the deposition. It is easy to conceive of a nerve control of the blood supply but it becomes difficult to conceive of a nerve control of pure secretion of a gas from lower pressures to higher pressures. We must then look for mechanisms aside from mere nerve stimuli upon the so-called gland cells to call forth a pure secretion.

Fishes that deposit gases at all rapidly possess a gas gland always associated with a rete mirabile, the fundamental mechanics of which are the same in all fishes. Thus the mechanics of the rete mirabile-gas gland must be of prime importance. The carbon dioxide pressure of the swim-bladder is generally, if not always, higher than found in air which is only .03%. This, too, must be of prime importance. The question now is, how would it be possible for a nerve control of the blood supply to the swim-bladder to control the deposition of gas from the blood, a definite physico-chemical system, by the aid of the mechanism and conditions just described, that is, a rete mirabile-gas gland and a carbon dioxide partial pressure in the swim-bladder higher than the carbon dioxide tension of the surrounding water of the fish?

The arterial blood, which has been brought into equilibrium through the gills with the carbon dioxide and oxygen tensions of the water, the tensions of which generally approximate their respective partial pressures of the atmosphere bathing the water, enters the rete mirabile. It is then distributed between the very large number of capillaries of the rete mirabile. The blood is then gathered up by arterioles to arteries and is again distributed into arterioles and then into the epithelial capillaries of the gas gland thus raising the carbon dioxide tension of the blood to approximately that of its partial pressure in the swim-bladder which, as has been stated, is generally, if not always, higher than the carbon dioxide tension of the arterial blood. The oxyhemoglobin will, because of its physico-chemical characteristics, give up a large part of its oxygen. The blood being in a closed system with surroundings having a metabolism almost nil, the excess oxygen must go into solution in a plasma already in equilibrium with the external oxygen tension of the water. This automatically raises the oxygen tension of the blood. The oxyhemoglobin hemoglobin ratio is higher than it would have been had the oxygen tension remained constant. This excess oxyhemoglobin constitutes a back pressure against carbon dioxide. This is a well known fundamental physiological principle. To put it in the words of L. J. Henderson (1925, 1928), ". . . since carbonic acid influences the oxygen equilibrium in blood, oxygen must influence the carbonic acid equilibrium." All physiologists know that this was shown experimentally by Christensen, Douglas and Haldane (1913, 1914) when they pointed out that the carbon dioxide absorption curves of the oxygenated and reduced blood differed; that is, oxygen drives carbon dioxide out of the blood. Again the swim-bladder-gas gland is a closed system. There is no avenue of escape for either augmented oxygen or the carbon dioxide that is driven out by the oxygen in the blood except into the lumen of the swim-bladder. The carbon dioxide could not return to the swim-bladder since it entered the blood from a higher pressure, and as a matter of fact, the total oxyhemoglobin is less in amount after the entrance of the carbon dioxide into the blood than before, since its very entrance into the blood has reduced a given definite amount of oxyhemoglobin to hemoglobin (Bohr, 1904, and Bohr, Hasselbalch and Krogh, 1904, and subsequent workers). Hemoglobin is a weaker acid than oxyhemoglobin. Thus the blood would hold the carbon dioxide with a greater tenacity than before. And on the other hand, if any of the oxygen for any reason should enter the swim-bladder, as all observations show that at times it does, the tenacity with which the blood would hold the carbon dioxide at the given pressure would still be increased since more of the oxyhemoglobin would have been reduced to hemoglobin by the escape of oxygen into the swim-bladder. Another drain on the carbon dioxide of the system would be the obvious escape of carbon dioxide through the final outgo of venous blood from the gas gland-rete mirabilia system.

The carbon dioxide in the swim-bladder would be dissipated by entering the blood during its passage through the capillaries of the gas gland. This is in opposition to observed facts. Carbon dioxide and oxygen are both found in the swim-bladder at higher partial pressures than their respective tensions of the arterial blood entering the rete mirabile. The blood as a physico-chemical system cannot account for the deposition of oxygen or carbon dioxide into the swim-bladder. In short, a man cannot lift himself to a higher elevation by pulling upon his boot straps. To put it in other terms, "It is impossible for a self acting machine, working entirely independently of any external agency to convey heat from one body to another at a higher temperature." That is, gases cannot diffuse through membranes or otherwise from a lower to a higher tension or escape from a liquid or through a membrane from a lower to a high pressure. The augmentation of the oxygen tension brought about by the reduction of the oxyhemoglobin to hemoglobin by the carbon dioxide is not sufficient to cause the oxygen to pass by simple diffusion into the swim-bladder of deep water fishes. However, a .30% of an atmosphere of carbon dioxide is ten times normal, 3.0% is one hundred times normal, and 30.% is one thousand times normal. The five atmospheres of carbon dioxide observed (Haldane, 1898) in a swim-bladder of a deep water fish would be more than 15,000 times normal. However, even if all the oxygen were liberated it would not raise the oxygen tension to 127 atmospheres which was the pressure of the oxygen in the swim-bladder of the fish taken at a depth of 4,500 feet (Haldane, 1898). Another explanation must be found. A source of energy and a mechanism with the ability to utilize this energy to raise the tensions of carbon dioxide and oxygen sufficiently high to deposit these gases into the swim-bladder against this tremendous pressure must be found before an adequate explanation for the deposition of gases into the swim-bladder against pressure can be found.

The diffusion pressure of the oxygen toward the lumen of the swim-bladder is now very much augmented. The blood leaving the capillaries of the gas-gland has an augmented oxygen tension, not being able to give up all of its oxygen to the swim-bladder by simple diffusion. The oxygen tension of blood that is actively depositing oxygen into the swim-bladder cannot be less than the oxygen partial pressure in the swim-bladder. Gases in simple solution diffuse from a higher to a lower tension, a fundamental principle expressed in Henry's and Dalton's laws. Campbell (1931) states, "It is generally—but not always—admitted that the passage of gases through the tissue is regulated by the laws of diffusion." There are no reasons to suppose that gases in simple solution in passing through a permeable membrane follow any other than the laws of diffusion through a liquid. The direction of diffusion would follow these same laws of diffusion even though the gases should go into chemical combinations with the permeable membrane pro-

vided the gases enter on the one side and emerge from the other side of the permeable membrane in simple solution. The carbon dioxide tension of the blood leaving the capillaries of the gas gland would at least approximate the carbon dioxide partial pressure of the swim-bladder, since the carbon dioxide is free to diffuse from the swim-bladder to the blood in the gas gland capillaries. The blood having been equilibrated with a carbon dioxide tension greater than the carbon dioxide partial pressure of the atmosphere has become modified as it enters the venules leaving the gas gland, *i.e.*, its carbon dioxide and oxygen capacities have both been decreased (see Table XXV). Again the venules being a closed system, the tension of both the oxygen and carbon dioxide are further augmented as the blood passes into the venous capillaries distributed among the arterial capillaries of the rete mirabile. Here, the venous and arterial blood travel side by side, a distance of eight or so times the length of an average capillary; but of course, the flow is in opposite directions, which is more advantageous as will be explained later. The venous blood now having higher carbon dioxide and oxygen tensions than the arterial blood, carbon dioxide and oxygen will both diffuse from the venous blood into the arterial blood, thus building up the carbon dioxide and oxygen tensions of the arterial blood which is on its way to the capillaries of the gas gland. These tensions are further built up in the arterioles by the blood being modified, as just described. The carbon dioxide carrying capacity of the venous blood being lower than the arterial blood, there will be a greater tendency for the carbon dioxide, its tension being augmented as already explained, to pass from the venous blood to the arterial blood and be carried back to the gas gland capillaries. Thus the carbon dioxide and oxygen tensions of the arterial blood will tend to be built up higher than their respective tensions of the water external to the fish. These tensions will be still further augmented since the arterial blood is now modified by the high carbon dioxide tension, thus its carrying capacity for oxygen and carbon dioxide will be lowered. By repeated circuits (Fig. 27) the oxygen and carbon dioxide tensions will increase until their tensions in the blood in the gas gland reach equilibria and will finally surpass their partial pressures in the swim-bladder. Figure 28 shows the possibility of the building up of an enormous pressure of oxygen in the swim-bladder although the partial pressure of the carbon dioxide in the swim-bladder be only 3% of an atmosphere. The blood with a carbon dioxide tension of 3% of an atmosphere would have to have a much higher oxygen tension to hold the 16 volume per cent oxygen that the unaltered blood contains at a carbon dioxide tension of .03% of an atmosphere and an oxygen tension of only 20% of an atmosphere (see Figure 28) not taking into consideration the augmented oxygen tension. In fact, if the capacity of the blood were reduced by being modified to below 16 volume per cent the liberated oxygen would have to go into simple solution in the plasma as the

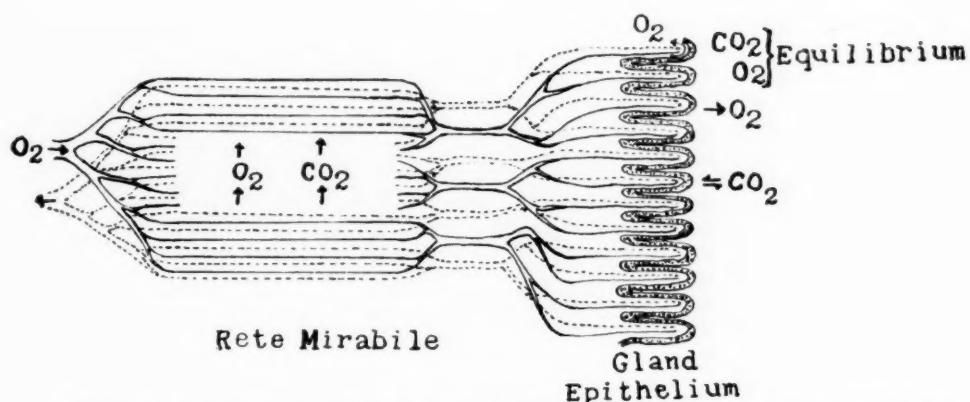


Figure 27 is a graphic representation of the rete mirabile-gas gland mechanism. The pointers indicate the direction of diffusion of oxygen and carbon dioxide from the venous capillaries to the arterial capillaries of the rete mirabile.

unaltered hemoglobin would already be approximately 100% saturated. Thus the oxygen tension of the blood would be more rapidly augmented during the continuous circuits.

One of the difficulties in handling fish blood is to prevent the red blood corpuscles from breaking down. A 20 lb. common sturgeon, *Acipenser sturio* Linnaeus, was taken from a live box in the river, wrapped in a moist cloth and carried twenty-five miles by automobile to our laboratory and placed in our artificial pond. It never completely recovered after being placed in the water. When blood was drawn directly from the heart by the red blood corpuscle pipette of the Thoma-Zeiss haemacytometer into Hayem's solution it was found that 65% of the red blood corpuscles were broken down as evidenced by the nuclei which had not disintegrated. This observation was verified by making a blood smear. A nine pound carp brought to the laboratory under adverse conditions a distance of over fifteen miles was in a dying condition and showed no signs of recovery when placed in our laboratory pond. When the blood was drawn from the heart it was shown that practically all the red blood cells were broken down and the blood was a brown cherry red. A second ten pound carp had been kept in our laboratory pond for more than two months and as far as could be observed was in good condition. It was placed in a galvanized can of water to which carbon dioxide was added, and kept for about three hours. At the termination of the experiment the carbon dioxide tension of the water was approximately 31.54 mm Hg. and the oxygen content 2.0 ml per liter. It showed a marked increase in the rate of respiration there being 65 per minute. The fish showed gradual weakening. The blood, a dark cherry red, was drawn directly from the heart and examined immediately. It was found that 64% of the red blood corpuscles were broken down. This leaves no doubt that the red blood corpuscles can through adverse conditions be broken down in the blood stream of fishes. One of these adverse conditions is high carbon dioxide tension of the blood.

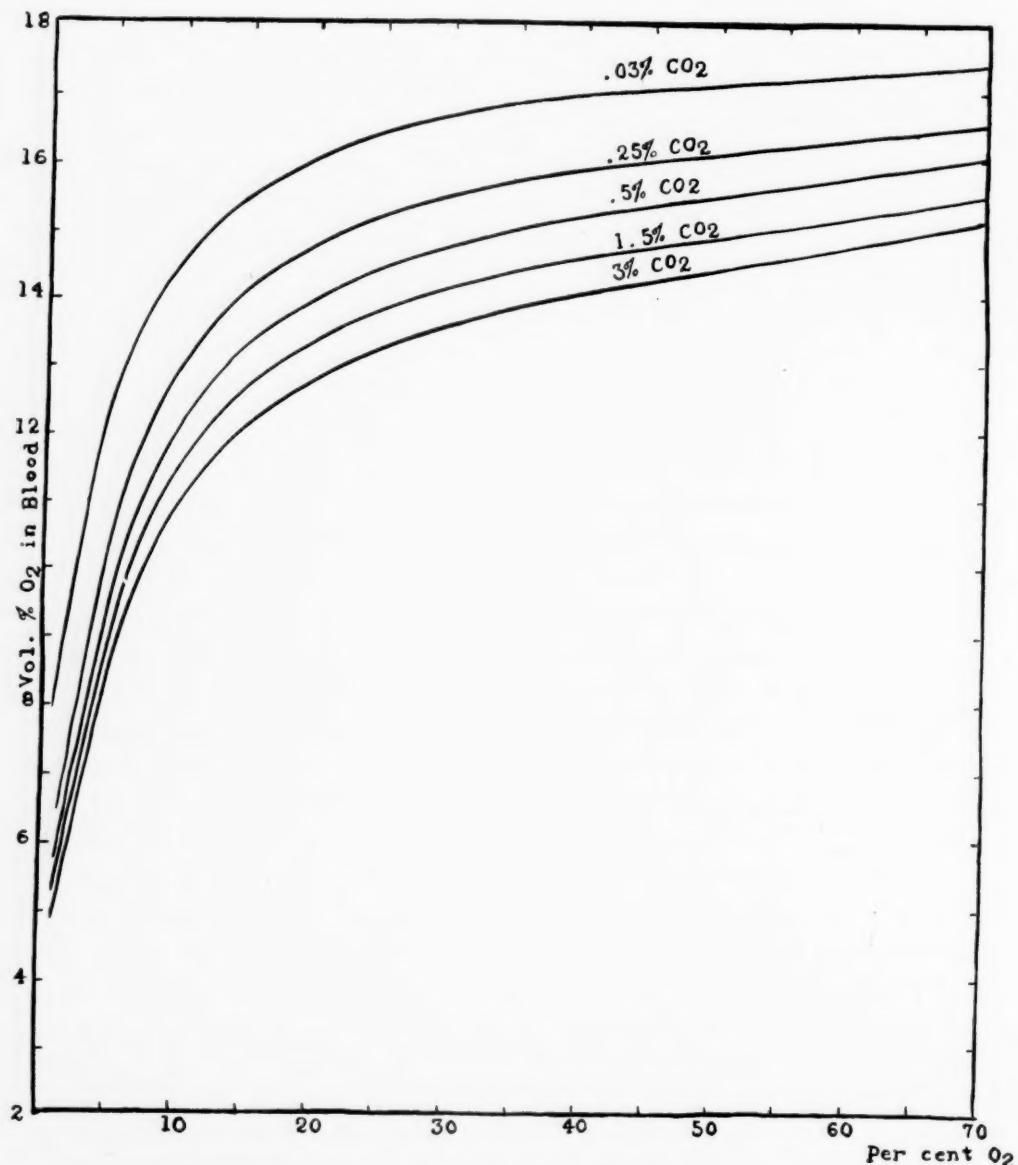


Figure 28 are the oxygen dissociation curves expected of the blood of the black horse, *Cyclopterus elongatus* (Le Sueur), from the oxygen capacity of the blood when equilibrated with 20% oxygen and with percentages of carbon dioxide indicated on each of the curves. See the oxygen capacity and the carbon dioxide dissociation curves of the blood of the black horse Figure 14 and Table XVII.

It had already been shown by Powers and Shipe (unpublished) that the breaking down of red blood corpuscles in drawn blood of the fishes could be accelerated by equilibration at even low carbon dioxide pressures. There could have been no substance synthesized by a gas gland cell and poured into the blood as the blood was outside the body of the fish. Thus, there is no need to postulate a toxin other than carbon dioxide to account for the

breaking down of the red blood corpuscles in the blood of the gas gland-rete mirabile in which the broken down red blood corpuscles were actually observed by Reis (1906), Nusbaum (1907) and Woodland (1911), and others before them. However, Woodland (1912) seems to have modified his views.

In view of the fact that carbon dioxide at pressures higher than ordinarily met with by the fish will both break down red blood corpuscles and reduce the carbon dioxide and oxygen carrying capacities of the blood, the mechanism and the source of energy necessary to deposit carbon dioxide and oxygen into the swim-bladder of the fish at very high pressures are obvious. The explanation becomes very simple.

Hemoglobin in solution is a less efficient oxygen carrier than hemoglobin in an organized red blood corpuscle. This is true especially at the higher oxygen tensions. This and the fact that the blood is modified in a way which reduces its carbon dioxide and oxygen carrying capacities show plainly, with the structures associated in the gas-gland rete mirabile, the mechanism and, with the source of energy necessary to make the deposit, how carbon dioxide and oxygen can be deposited into the swim-bladder at pressures higher than their respective tensions in the arterial blood. The enormous pressures found in the swim-bladders of deep sea fishes are not exceptions. However, the breaking down of the red corpuscles is not a necessary factor in the deposition of gases into the swim-bladder.

In our own laboratory another proof has been found that the blood of fishes is altered when brought under adverse conditions. By a microscopical examination of the drawn blood it was found that crystals very quickly appeared on the inside of the red blood corpuscles. As time progressed these crystals changed forms (see Figs. 20 to 26, p. 438) and in some bloods they passed through a regular cycle. In some of the bloods examined crystals were either already formed in the red blood corpuscles in the blood stream of the fish or they were formed during the time necessary to carry out the manipulations to make a microscopical examination of the blood. The most beautiful of the form of crystals shown (Fig. 22, p. 438), were found in the blood vessels of a (dead) fish from which we had drawn blood an hour or so before. The nature of the crystals has not been determined. The observations can mean but one thing and that is the blood is very rapidly modified when brought under adverse conditions. This is true of the fish itself as evidenced by the difficulty with which fresh fish are handled. The fact that crystals were found in the blood vessels of a fish is sufficient proof that the modification of the blood to form crystals was not brought about by the addition of chemicals used to prevent the coagulation of the blood.

The explanations given above are sufficient to make plain how carbon dioxide and oxygen are deposited in the swim-bladder. It does not explain, however, the deposition of inert gases,—nitrogen, etc. Haldane (1898) calls

attention to the observations of Schloesing and Richard (1896) where the swim-bladder of a fish taken at a depth of approximately 4,500 feet, comparable to 150 atmospheres mechanical pressure, contained 85% oxygen and 12% nitrogen and argon. From this it follows that the partial pressures of the oxygen was 127 atmospheres and that of the nitrogen and argon 18 atmospheres. Carbon dioxide made up the remaining 5 atmospheres. In this case we have the deposition of oxygen, nitrogen, argon and presumably all gases contained in the atmosphere above water. All workers from the time of Bohr (1894) have held that the so-called secretion was not pure oxygen, but is composed of the atmospheric gases high in oxygen.

Nitrogen, argon, helium, etc., are almost, if not entirely, inert gases. Inert gases being in solution can pass through permeable membranes by diffusion only. The diffusion pressure is determined by the tension of the gas in solution (Graham's, 1842, and Exner's, 1875, laws) which is the equivalent of the partial pressure with which it has been brought into equilibrium, this being the gaseous partial pressure found in the atmosphere.

Henry's Law is "The amount of gas which a liquid will dissolve is directly proportional to the pressure of the gas. This holds for all gases which do not unite with the solvent." Dalton's Law states that "The pressure of a mixture of several gases in a given space is equal to the sum of the pressures which each gas would exert if confined by itself in that space." Or as it is sometimes stated, "Each gas exerts its partial pressure independently of all other gases present." Thus, the amount of any gas dissolved in a solvent depends upon two factors, one the pressure of the gas equilibrated with and the other the dissolving power of the solvent. It follows from these laws that the tensions of these inert gases entering the gas gland could never be greater than their partial pressures of the atmosphere with which the water external to the fish was equilibrated, with the exceptions due to changes in temperature. Another explanation aside from simple diffusion for the deposition of inert gases into the swim-bladder against higher pressures of these gases must be found. Since inert gases do not form loose chemical combinations with substances in blood there could be no augmentations of their partial pressures within the blood stream as with oxygen and carbon dioxide. For an explanation of the deposition of these inert gases into the swim-bladder, one must look to the behavior of gases in simple solution, that is, the laws of solution of gases and the laws of diffusion of gases in simple solutions. In order to promote a better understanding of the principles of Henry's and Dalton's laws a few simple experiments were performed (see p. 389) to demonstrate the fact that the tendency of gases to form bubbles beneath the surface of a fluid is not determined by the gaseous complex or the partial tension of any one gas but is entirely dependent upon the total of all partial tensions. In short, bubbles will not be formed until the sum or the total of

all gaseous partial tensions is greater than the mechanical pressure, *i.e.*, the hydrostatic (the column of water) plus the atmospheric pressure above the water. Just as soon as this total is sufficiently greater than the mechanical pressure to overcome other opposing physical forces tending to hold the gases in solution or preventing the gases from passing into a gas phase, bubbles will be formed. The gaseous complex and the respective partial pressure complex of the gases in the bubbles will be the same as the gaseous complex in solution and their respective tension complex (Henry's Law). As was shown by Reis and Nusbaum (1905), Nusbaum (1906, 1907), Woodland (1911) and others, gas bubbles are formed in the gas glands. These bubbles will contain all the gases in solution and will be made up predominantly of oxygen since it is the oxygen partial tension that has been augmented (see above) to the point that the total gaseous tension is greater than the mechanical pressure. Thus the gas deposited into the swim-bladder of a fish would not be pure oxygen, which is the conclusion arrived at by all workers, but of oxygen plus all other gases in solution in the blood and in turn in the water external to the fish. Traube-Mengarini (1889) has shown that hydrogen can be deposited into the swim-bladder when this gas is dissolved in the water surrounding the fish. It then follows that the inert gaseous complex in the swim-bladder would be the same as that of the atmosphere with which the water had been equilibrated regardless of the pressure against which they were deposited. This should be true since these inert gases are deposited in the swim-bladder against pressure by the gas bubbles breaking into the lumen of the swim-bladder. It should no longer be doubted that the structures observed by Reis, Nusbaum and Woodland were the remnants of gas bubbles. The behavior of gases in solution tells us that this is the only way that gases, regardless of their immediate origin, can get into the swim-bladders of deep sea fishes where its gaseous pressure must equal the hydrostatic pressure plus the atmospheric pressure above the fish. An exception in the case of oxygen will be mentioned later. Gases will not diffuse up hill. If the total gaseous tension is raised above the hydrostatic plus atmospheric pressure—the gaseous pressure in the swim-bladder—the gases will pass from the solution phase to the gas phase. The gas bubble will contain all gases that are in solution in direct proportion to their respective tensions. Where the inert gases in the swim-bladder have been determined, they have been found to be present in the same proportions that they are found in the air. Schloesing and Richard as early as 1896 pointed out the fact that nitrogen and argon were present in the swim-bladder of deep water fishes in the same proportions found in air although the total of their partial pressures (Haldane, 1898) was 18 atmospheres. How can this enormous pressure of these inert gases be built up? The explanation is simple. These inert gases are carried into the swim-bladder bit by bit by the gas bubbles formed in the gas glands and passed into

the lumen of the swim-bladder where they break and give up their inert gases regardless of the inert gaseous partial pressures in the swim-bladder. But the actual partial pressures of the inert gases in the bubble cannot be greater than their respective partial pressures of the atmosphere with which the water external to the fish was equilibrated. Thus it follows that since the partial pressure of the oxygen approaches the mechanical pressure which is the same as or at least approximates the gaseous pressure of the swim-bladder, the partial pressures of the inert gases would never be raised above their respective pressures found in the atmosphere with which the water was equilibrated. That is, it is impossible because of the behavior of gases for an inert gas to be deposited into the swim-bladder by simple diffusion or by the formation of gas bubbles which break into the lumen of the swim-bladder at pressures higher than their respective pressures in the atmosphere.

All workers who have made the observation report that the walls of the swim-bladder, if not impermeable, are almost impermeable to gases. Thus gases do not enter or escape from the swim-bladder except through the blood stream. The blood supply to the walls of the swim-bladder, which in some cases is rather copious, aside from the rete mirabile-gas gland association is through the ordinary artery-capillary-vein mechanism. That is, the oval contains a blood supply quite different from that of the gas gland-rete mirabile mechanism. It follows from all the physical laws of diffusion and osmosis that all gases contained in the swim-bladder at these enormously high pressures would tend to diffuse into the blood, the arterial-capillary-venous system, and be carried to the gills where all gases including the inert gases would be given off into the water. This especially would be the case with the inert gases since the inert gases in the blood would already be in equilibrium with those of the water unless there was sufficient deposition of these gases to deplete the blood down to the point that the added gases would not raise the tensions up to those of the water. This would have to be the case in some instances, *i.e.*, venous blood from the rete mirabile, since there is an actual stacking up of the inert gases in the swim-bladder. The carbon dioxide and oxygen would also diffuse into this blood, *i.e.*, in the oval, and be carried out of the swim-bladder. But in addition to being carried in mechanical solution, as is the case with the inert gases, they would also be carried out in chemical combinations. The result is that the carbon dioxide and oxygen would be carried out of the swim-bladder in the process of emptying more rapidly than the inert gases. This would tend to leave the inert gases behind. Thus the inert gases in the swim-bladder would be built up to higher partial pressures than their respective tensions in the blood stream supplying the rete mirabile-gas gland system. If all the carbon dioxide and oxygen should be removed from the swim-bladder, the inert gases would have a total pressure exactly equal to the hydrostatic plus the atmospheric pressure above the fish unless

the musculature was exerted to compress or expand the volume of the swim-bladder. In that case the difference in hydrostatic plus atmospheric pressure and the pressure inside the swim-bladder is exactly that exerted by the musculature. It would be no more nor less. Finally, in all of these forces acting, there would be a tendency to reach equilibrium which would approximate, in the case of the fish taken by Schloesing and Richard at a depth of about 4,500 feet, a mechanical pressure of about 150 atmospheres, oxygen 127 atmospheres, nitrogen and argon 18 atmospheres, and carbon dioxide 5 atmospheres.

An unequal distribution on the two sides of a structure or even a membrane does not mean secretion in the true sense of the term. Instead, I have used the term deposition. As has been stated it is a system tending to reach equilibrium. This point of view is well illustrated by Donnan's Equilibrium, in which hydrogen ions are unevenly distributed on the two sides of a membrane permeable in both directions to hydrogen ions. The hydrogen ions do not pass against pressure in either direction. If there were a continuous removal on the other side, there would be a continuous flow in one direction. In the end, substances secreted by the kidneys and other so-called secreting glands will be found to move always toward a lower pressure. Such actions should thus be called depositions. The secretion of oxygen by the lungs perhaps remains an open question. On the other hand, the deposition of oxygen into the swim-bladder cannot be used as an illustration of a method by which the lungs might secrete oxygen, since there are no structures in the lungs comparable to the rete mirabile. On the other hand, in the blood supply to the pulmonary capillaries, the arterioles and venules do not come into relation until they have reached considerable size (Cunningham, 1920). This is in direct opposition to the relations of the blood vessels in the gas gland-rete mirabile mechanism.

If we look for a cause—or at least for an advantage—of this particular arrangement of the pulmonary arterioles and venules, it can be found. The arrangement prevents to a minimum the diffusion of oxygen from the loaded arterial blood to the venous blood, and the unloaded carbon dioxide of the venous blood to the arterial blood.

Fishes without a gas gland-rete mirabile can deposit oxygen into their swim-bladder. Some of these fishes that have been examined in our own laboratory show arteries and veins, arterioles and venules down to small sizes running parallel in groups and closely approximating one another. The deposition of oxygen would be somewhat inefficient, but could be accomplished by this structural relation of the blood vessels.

The only significance of the gaseous complex of the swim-bladder is that it expresses a dynamic equilibrium, which the science of physics has long recognized as fundamental and which we as biologists often talk about but

seldom follow to its logical end. This dynamic equilibrium is a physiological-physico-chemical equilibrium. The explanation is purely mechanistic, and the gas complex, as stated, is purely an expression of equilibrium. One of the factors tending to cause variations in the gaseous complex of the swim-bladders of the same species at the same depth, and variations at different times even of the same fish at the same depth, is the percentage of gas that has been deposited into the swim-bladder in the form of bubbles: another is the amount of carbon dioxide and oxygen that has been removed by the oval. In deep water fishes, where the inert total gaseous partial pressures in the swim-bladder are greater than their total partial tensions of the water, the diffusion tension of the oxygen in the rete mirabile-gas gland system could be in the direction of the lumen of the swim-bladder before the gaseous total partial tensions would equal this gaseous total partial pressures within the swim-bladder. The oxygen would then pass by simple diffusion into the swim-bladder. This point would be reached when the total partial tensions equal the gaseous total partial pressures of the swim-bladder, minus the total partial pressures of carbon dioxide and the inert gases in the swim-bladder, minus the total partial tensions of the same gases in the blood of the rete mirabile-gas gland system. This is merely a mathematical way of saying that just as soon as the oxygen tension of the rete mirabile-gas gland system is greater than the oxygen partial pressure in the swim-bladder, oxygen will pass by simple diffusion into the swim-bladder, and that this can take place before the gaseous total partial tensions of the rete mirabile-gas gland system reaches a value equal to the gaseous total partial pressures in the swim-bladder.

The deposition of the gas into the swim-bladder and the emptying, at least among fishes with closed swim-bladder, are without doubt under nerve control, as shown by Moreau (1876) and Bohr (1894). The fish no doubt responds in such a way as to control the blood supply to the rete mirabile-gas gland to deposit, and the blood supply to the oval capillaries of the swim-bladder wall to resorb the gases from the swim-bladder and thus keep the fish in adjustment to its environment. In this case, as in all others, when the rapidity in the changes in environmental factors, such as a too rapid raising or sinking of the fish is sufficient to outrun the physiological-physico-chemical adjustment, derangement is the result. Such was likely the case in the gas-bloated fish discovered by Greene (1924, 1924b) floating helplessly on the surface of Monterey Bay. Hall (1924) reports an instance in which the swim-bladder was actually distended by a rapid change in the carbon dioxide in the water. Greene (1924b) reported another interesting observation. The swim-bladder of a large specimen of *Porichthys notatus* was emptied as completely as possible and refilled with atmospheric air under a pressure of six feet of water. Two days later a sample of gas from the swim-bladder "showed an oxygen percentage of 31.3. Four days later the oxygen of the

refilled bladder gave a percentage of 58.8, and again, 8 days later, 68.1 per cent. In the drawing of each of these samples the gas was as completely emptied as possible, and after each withdrawal the fish lost the power of vocalizing. The conclusion is obvious, that atmospheric air can be resorbed from the air-bladder in such a way as to leave a preponderance of oxygen." Thus the inference is that the inert gases can be resorbed so as to leave lower internal inert gases percentage composition (pressure) than the percentage inert gases (tension) complex in the water surrounding the fish. This, of course, is supposing that the inert gases in solution in the water surrounding the fish are in equilibrium with those of the atmosphere, and this is most probable. This is in opposition to a theory of simple diffusion of inert gas that has been suggested in this paper. The analyses of gas samples from the swim-bladders of *Porichthys notatus* under various conditions show without exception that the per cent of oxygen is greater than that found in the atmosphere (Greene, 1924a). Any hydrostatic pressure experienced by the fish still increases the difference between the oxygen percentage in the swim-bladder and the oxygen percentage in the atmosphere. Thus the high oxygen percentage content of the swim-bladder is a normal condition in *Porichthys notatus*. If the resorption of inert gases at a percentage lower than that found in the atmosphere cannot be explained by simple diffusion, a pumping mechanism must be postulated. Is there a way out of the dilemma? Inert gases do not go into chemical combinations. The pumping mechanism cannot be chemical and thus must be physical. A physical pump would be quite simple provided inert gas could be brought from the gas gland into the swim-bladder by bubbles of gas breaking into its lumen at one pressure and the internal pressure raised, allowing the inert gas to pass out by simple diffusion and then again the pressure lowered. The inert gas could then have an inert gas percentage composition less than that of the atmosphere. Does *Porichthys notatus* (the California singing fish) have such a physical pump? In this fish a "noise or vocalization is produced by sudden unequal pressures on the gases of the two chambers of the swim-bladder." "The pressure is accomplished by the contraction of the air-bladder muscle." The muscles bringing about this pressure are as described by Greene (1924b) "rather heavy masses" and "striated." The inert gases are thus not diffusing from a lower pressure to a higher tension but from a higher pressure to a lower tension when they leave the swim-bladder. Thus the internal pressure of the swim-bladder of *Porichthys notatus* is always greater than the hydrostatic plus atmospheric pressure since normal striated muscles are always under tension. Thus the inert gases could have percentage compositions less than in the atmosphere and under the conditions that they exist still have partial pressures greater than in the atmosphere. It is a fact long known that the oxygen percentages in the swim-bladders of fishes increase with the depth at which the fishes are found.

Again inert gases cannot, except through simple diffusion and on the inside of gas bubbles composed mostly of oxygen, enter the swim-bladder. The pressure of these inert gases on the inside of these gas bubbles cannot be greater than their respective tensions in the blood. They cannot leave the swim-bladder except through simple diffusion. Thus the actual internal inert gaseous pressures, not percentages of composition, would tend to be greater than their tensions in the water surrounding the fish, since oxygen enters and also leaves the swim-bladder readily. The inert gaseous pressures as stated would always tend to be higher than their external tensions with possibly two exceptions. First, if the swim-bladder should empty by bubbles entering the blood from the swim-bladder, the inert gases could be carried out regardless of their pressures inside the swim-bladder. This could not happen, however, unless there was a pressure through muscular contraction to bring about a total pressure higher than hydrostatic plus atmospheric, making formation of bubbles possible. Second, if the inert gases had at a high external pressure in the swim-bladder reached or approximated equilibrium with the inert gaseous tensions outside the fish, and the swim-bladder could be allowed to expand, increase in volume, the inert gaseous internal pressures could then be less than their external tensions. This could happen only if a fish should suddenly move into shallower water or if the musculature of the swim-bladder, for example in *Porichthys notatus*, should suddenly relax after a long period of contraction. Neither of these conditions could last, since there would be new points of equilibrium which would sooner or later be approximated. In all fishes which are able to deposit gases into their swim-bladders at all rapidly, their emptied swim-bladders refill with gases composed primarily of oxygen. The explanation of this is very simple. Oxygen can be deposited more rapidly than inert gases.

Another advantage of the mechanical arrangement of the rete mirabile is that if gas bubbles were formed and carried beyond the gas gland into the venous system, they would be resorbed in the rete mirabile and not run any chance of clogging the capillaries of the liver, a closed system, with gas bubbles. That is, a part of the gases pass from the venous capillaries of the rete mirabile to the arterial capillaries so that the venous blood leaving the rete mirabile would have a gaseous total tension less than the mechanical pressure of the blood. This is made certain since the blood is flowing in opposite directions in the venous and arterial capillaries in the rete mirabile.

From the above discussion it follows that the primary function of the swim-bladder is, as first suggested by Needham (1667), hydrostatic. All other functions, as sound production, respiration, etc., are secondary. It is quite difficult to see how a fish, since all of the blood flowing from the swim-bladder must of necessity pass through the capillaries of the gills before supplying the tissues, could use its swim-bladder as an organ of respiration. The

oxygen in the blood at higher tensions would, when reaching the capillaries of the gills, pass out into the water unless the gills by closure of the opercula and mouth could be entirely cut off from the surrounding water. In this case the elimination of carbon dioxide by the gills would be inhibited. In short even with a pneumatic duct, it is mechanically impossible for the swim-bladder to perform the function of oxygen absorption and the gill, the function of carbon dioxide elimination as suggested by some. Also, at the present time there seems to be no satisfactory physiological-physico-chemical explanation for "secreting" oxygen in either direction without a mechanism comparable to the rete mirabile-gas gland mechanism. It seems more reasonable to suppose, looking at the economy of nature, that fishes with pneumatic ducts that "break" in water low in oxygen swallow the air, and both swim-bladder and gills perform the function of gaseous exchange as do lungs, unless the gills are cut off entirely by the closure of the opercula. In that case the gills would be obsolete, and the swim-bladder would act entirely as lung.

Finally it must be determined how the first gas gets into the swim-bladder. Ledebur (1928) performed experiments in Winterstein's laboratory in which he found that if certain fishes in their development were not allowed to swallow air before the closure of the ductus pnuematicus they would perish without the swim-bladder ever being filled with gas.

There are two possible ways in which the swallowing and forcing air by way of the pneumatic duct into the swim-bladder could initiate the process of gas deposition into the swim-bladder. If the air swallowed had a higher oxygen and a lower carbon dioxide pressure than their respective tensions in the water surrounding the fishes, the conditions would be such that gas secretion could be brought about. This is generally the case except where vegetation in the presence of sun-light predominates and even this would not be the case during night. Oxygen would be absorbed from the swallowed air, and carbon dioxide would be liberated into the swim-bladder comparable to lung, because of the nature of blood as a physico-chemical system. Or if the carbon dioxide did not escape into the swim-bladder, its tension would be raised in the blood as it is carried to the rete mirabile. Since, according to Krogh (1929), carbon dioxide diffuses about twenty times as rapidly as oxygen, more carbon dioxide than oxygen proportionately would pass from the venous capillaries to the arterial capillaries in the rete mirabile and be carried back to the swim-bladder. To put it another way, oxygen would escape proportionately more rapidly from the swim-bladder through the veins than would carbon dioxide. This, as in the case of the inert gases, would tend to build up the percentage of carbon dioxide. Since the mechanical pressure on the outside of the swim-bladder determines the pressure of the gas within the swim-bladder, this would tend to build up the carbon dioxide partial pressure, which, just as soon as it becomes higher than the carbon dioxide tension of the arterial blood, would start the deposition of oxygen and inert gases.

The above is perhaps a satisfactory explanation but not the probable explanation for the initiation of the first deposition of gas into the swim-bladder of fishes. It might be a factor in shallow and surface water fishes, but it could not hold at all with deep sea fishes as they never come to the surface of the ocean to swallow air. A more probable explanation—at least with deep water fishes and perhaps a factor with other fishes—is that the carbon dioxide originates within the swim-bladder. There are tissues or at least organic materials (Ledebur, 1928) inclosed within the tissues that are destined to form the walls of the swim-bladder. These disintegrate, liberating carbon dioxide, which in itself is sufficient to initiate the deposition of gas by the gas gland. The swallowing of air or otherwise contaminating with bacteria would stimulate the disintegration to liberate carbon dioxide. This is an hypothesis, but a most probable hypothesis. The difficulty would be the prevention of bacterial infection rather than a bringing about of infection.

The explanation given for the deposition of gases into the swim-bladder is in reality simple diffusion. From the behavior of gas as has been pointed out, the deposition must be primarily through bubbles. Is the structure of the epithelium of the gas gland such as would permit a passing of bubbles into the lumen of the swim-bladder? Nusbaum (1906) has described from his own observations and from the observations of others passages in some forms quite definite leading between the cells of the gas gland epithelium into the lumen of the swim-bladder. He states that in certain cases these even pass between the cells of the walls of the capillaries. He further states that gas bubbles have been observed in these passage ways.

A very important question now arises. That is, what is the force and its source that pushes the bubble through this passage way into the lumen of the swim-bladder? The bubble of necessity must pass into the swim-bladder, or there would be no deposition of gases. There must be some force that is not in equilibrium. But the gases in the bubble and in the swim-bladder are both in the gas phase under the same mechanical pressure and thus should be in equilibrium.

The behavior of gases in solution tells us that the total gaseous tension must be greater than the mechanical pressure or bubbles will not be formed. Solutions are supersaturated before crystallization is initiated. Water vapor is supersaturated before condensation, and so on. It follows that when bubbles are formed, the immediate vicinity is supersaturated with gases. The gases will then diffuse into the bubble with a total pressure equivalent to their total tensions. Thus the bubble will tend to build an internal pressure greater than the pressure within the swim-bladder. The bubble would then tend to move in the direction of the lesser pressure. The lesser pressure is in the direction of the swim-bladder.

It might still be argued that since the gases in solution pass into the bubble

this would tend to diminish the internal gaseous tensions in the immediate vicinity and thus come into equilibrium with the mechanical pressure on liquid. The internal gaseous tension approaches, but obviously it could never be less than the mechanical pressure or the gases would go back into solution. Just as soon as a gas bubble is formed there is the addition of water vapor pressure. Water vapor pressure is determined by the temperature and is independent of all or any external mechanical pressures. Thus, the growing bubble is always against the resistance of the intracellular passages. Again, the total of all gaseous pressures, including water vapor, in the swim-bladder is equal only to the mechanical pressure upon the swim-bladder. From these facts it follows that the pressure of a gas bubble would under all circumstances be from the blood stream to the lumen of the swim-bladder. This is a sufficient explanation as to why the gas bubbles would always of necessity pass toward the lumen of the swim-bladder.

XI. GENERAL SUMMARY

BY EDWIN B. POWERS

1. The physiology of respiration in fishes is the same as in lung breathing animals. Oxygen is absorbed and carbon dioxide is eliminated through membranes of the respiratory organs in each by the process of osmosis or diffusion. The water is the external medium in the one and the fluid covering the alveolar membrane is the external medium in the other.

2. The organs of respiration in fishes are more or less open systems. On the other hand, the lungs of lung breathing animals are more or less closed systems. This throws the burden of regulation more upon the internal environment, the blood, a physiological-physico-chemical system, in fishes than in lung breathing animals.

3. The rate and direction of diffusion through all membranes in both gill and lung breathers are determined by the partial tensions of the gases in solution in the fluids on the two sides of the permeable membrane and not by the actual amounts of the gases in solution in the two fluids. Gas like other substances cannot pass through a membrane by the process of osmosis unless it first be in solution in a fluid bathing the membrane and it pass into solution in a fluid on the opposite side of the membrane. Gases can pass out of or into either of these fluids into or out of a gas phase by simple diffusion. Gases in passing through permeable membranes follow the simple laws of diffusion of gases in solution. Gases in passing into or out of solution follow the simple laws of diffusion of gases from the gas phase to the solution phase and *vice versa*. In short, the behavior of a gas when associated with living organisms is not different from its behavior in non-living substances.

4. The oxygen and carbon dioxide partial tensions of natural waters which fishes inhabit do not have a definite ratio to each other as do their par-

tial pressures in the air. The relation of these two gases to each other in streams is strongly influenced by the relation of these gases in the waters at their sources. In fishes living in different natural waters is the same as living in atmospheres of different compositions.

5. The total amount of gases that can be held in solution in natural waters is determined by the total gaseous tensions and not by the partial tension of any one gas. The gaseous solution complex of a natural water is independent of the gaseous complex of the air except as diffusion takes place at the water-air surface. Gases will escape from a solution in the form of a bubble, that is, pass from a solution phase to a gas phase, when the total gaseous partial tensions exceed the mechanical at the point where the bubble is formed. This is true regardless of the gaseous partial tensions complex of the solution. The composition of the contents of the bubble is determined by the gaseous partial tensions complex of the solution and is independent of the composition of the atmosphere above the air water surface. The partial pressure of each gas in the bubble is determined by its partial tension in the solution as modified by its coefficient of diffusion.

6. The volume per cent of oxygen of venous blood of fishes increases with an increase in the oxygen tension of the water and *vice versa*. The carbon dioxide tension of the water has little or no effect.

7. The volume per cent of the carbon dioxide in the venous blood of fishes increases with an increase in the oxygen and with an increase in the carbon dioxide tension of the water and *vice versa*.

8. The number of red blood corpuscles is increased with a decrease in the oxygen and by an increase in the carbon dioxide tension of the water and *vice versa*.

9. The oxygen and carbon dioxide dissociation curves of fish blood are similar to those of mammalian blood. An exception to this is, that fish blood undergoes changes when equilibrated with carbon dioxide partial pressures higher than normal in which it loses capacity to carry oxygen and carbon dioxide. A carbon dioxide partial pressure is soon reached where a further increase brings about an increase in the oxygen holding capacity of the blood after which there is a further decrease in this capacity. The first change is due to the passing of the isoelectric point. The second is due to the continued loss in the capacity of the blood to carry oxygen as affected by its carbon dioxide tension.

10. The blood is modified, becoming more acid, when equilibrated with carbon dioxide partial pressures higher than normal, as indicated by a decrease in the pH of the blood when again equilibrated with air.

11. Drawn blood undergoes rapid changes during which crystals are formed. The forms of the crystals seem to pass through a more or less regular cycle. The first crystals are formed within the red blood corpuscles. The

first form will make its appearance in the blood within the fish that has met with adverse conditions.

12. The blood after being equilibrated at carbon dioxide partial pressures higher than the carbon dioxide partial pressure of the atmosphere loses in capacities to carry carbon dioxide and oxygen. When again equilibrated with atmospheric air the blood regains almost completely its original capacity to combine with oxygen. Under the conditions of our experiments the carbon dioxide combining power was never completely regained. The hemoglobin if modified, denatured or inactivated at higher carbon dioxide tensions apparently returns to normal, is reactivated or renaturated. If the carbon dioxide combining power of the blood returns to normal the reversal is much less rapid than in the case of the hemoglobin.

13. Red blood corpuscles will break down within the blood vessels of a fish that has met with adverse conditions, one of these adverse conditions being water having a carbon dioxide tension higher than that normally met with by fishes.

14. Gases are not secreted into the swim-bladder in the true sense of the term.

15. Gases are deposited mechanically or by simple diffusion into the swim-bladder after the total gaseous partial tensions have been increased in the blood of the rete mirabile-gas gland system above the total mechanical pressure upon the blood in the system, or when the partial tension of a gas in the blood of this system is increased above the partial pressure of the same gas in the swim-bladder of the fish. The mechanical deposition of the gases into the swim-bladder is by means of gas bubbles which are formed and pushed into the lumen of the swim-bladder as explained in the text. The total gaseous partial tensions of the blood is built up by the rete mirabile-gas gland mechanism through the modifications in the blood in which the oxygen and carbon dioxide capacities are decreased as explained in the text.

The explanation of the deposition of oxygen and carbon dioxide into the swim-bladder is chemical and physical and not hypothetical. The deposition of inert gases is purely physical only.

16. The swim-bladder cannot be used as an organ of respiration unless it be augmented by the gills as explained in the text or unless the gills are made obsolete and the swim-bladder used as lungs in air breathing vertebrates.

17. The secretion of oxygen into the lungs might still be an open question. But on the other hand, the deposition of gases into the swim-bladder cannot be used as an illustration of a mechanism by which this is accomplished.

18. It is our opinion that the so-called secretions by the kidneys, etc., are not true secretions, and in the end they will be found to be depositions and should thus be designated.

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CONCERNING CERTAIN PHYTOSOCIOLOGICAL
CONCEPTS*

By

STANLEY A. CAIN

Indiana University

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CONCERNING CERTAIN PHYTOSOCIOLOGICAL CONCEPTS

I. INTRODUCTION

Certain phytosociological concepts have received considerable attention from American ecologists. For example "frequency," especially Raunkiaer's so-called "Law of Frequency" has been discussed by Kenoyer, Gleason, Nichols, Hanson, Romell and others, while some other concepts have received very little attention. The present paper has for its purpose the clarification and organization of three groups of these concepts: (1) those relating to the organization of any one concrete example of a plant community; (2) the synthesis of the details of several comparable plant communities, *i.e.* different examples (stands) of the same community, into some more adequate picture of the association (if that be the community under study) in the abstract; and (3) the relation of the community, concrete or abstract, to other different communities. This paper owes a basic obligation to Braun-Blanquet and Pavillard's "Vocabulaire de Sociologie Végétale," yet has justification, for whatever repetition it contains, from the teaching point of view at least.

The descriptive phytosociology which recognizes the natural structural and dynamic aspects of a community is the best phytosociology. That, of course, is the aim of all descriptive work and is attained with varying degrees of success. Phenomena, varying as they do in anything so complex as vegetation, suffer in their description by various workers from a lack of uniformity, although any one piece of work, when viewed by itself, may be internally consistent, entirely adequate and wholly admirable. It is the aspiration of this paper, in a small way, by the presentation of a tabular form for compact assembly of data and the reviewing of concepts, many of them well-established, to help towards that goal where such scientific data and writings may be directly comparable, *i.e.* towards some degree of standardization in method and expression of results. Yet it must be recognized and constantly kept in mind that any "system" betrays its purpose when it does not reflect the natural truths of vegetation, no matter how fine it is philosophically. Pavillard (1923) has said, in connection with certain so-called laws of statistical phytosociology "Raunkiaer has refrained from indulging in such metaphysics, but his methods have been misused by some ecologists for certain untenable speculations. In respect to their "laws" the author agrees with Nordhagen's criticism and demands better philosophic considerations of the recommended methods." As Lutz (1928) has said of silviculture, "the silviculture which recognizes the tendencies of nature is the best silviculture."

II. CONCEPTS CONCERNING DATA DERIVED FROM QUADRATS (QUANTITATIVE)

There are three quantitative analytic concepts concerning the organization of a plant community. They are concerned with number, coverage, and uniformity of distribution, and are known respectively as abundance (density), dominance (coverage) and frequency.

ABUNDANCE DEFINED. Abundance is an appreciation of the relative number of individuals of each species entering into the constitution of the plant population of the territory under study. A scale of five degrees of abundance is suggested by Braun-Blanquet and Pavillard (1928).

- A1—species very rare in the community,
- A2—species rare in the community,
- A3—species not very abundant in the community,
- A4—species abundant in the community, and
- A5—species very abundant in the community.

DENSITY DEFINED. Density is the exact relation between the number of individuals of the same species observed on a certain territory and the extent of that territory, *i.e.* density is abundance on a unit area basis.

DOMINANCE DEFINED. Dominance concerns the extent (surface and volume) covered or occupied by the individuals of each species. In many-layered plant communities dominance must be evaluated for each stratum separately. To evaluate it one must be able to give the figures attributed to each species the exact significance indicated.

- Do 1—species covering 1-5% of the surface,
- Do 2—species covering 6-25% of the surface,
- Do 3—species covering 26-50% of the surface,
- Do 4—species covering 51-75% of the surface, and
- Do 5—species covering 76-100% of the surface.

FREQUENCY DEFINED. Frequency is concerned with the uniformity with which the plants of a species are distributed throughout a plant community. It is a statistical idea which is determined by arranging the complete floristic lists of a certain number of quadrats of equal dimensions disseminated as widely as possible throughout the extent of the individual stand of the community under study. The frequency of a species is expressed by the relation in per cent between the number of sample areas which contained it and the total number of such areas employed in the analysis. Frequency is usually expressed on a basis of five classes.

- Class A, F 1—species in 1-20% of the quadrats,
- Class B, F 2—species in 21-40% of the quadrats,
- Class C, F 3—species in 41-60% of the quadrats,

Class D, F 4—species in 61-80% of the quadrats, and

Class E, F 5—species in 81-100% of the quadrats.

The percentage is called the "frequency index" of the species.

DISCUSSION AND EXAMPLES OF THE PRECEDING CONCEPTS. It is important to recognize in any such ecological investigation the essential distinctness of the three concepts; number, area of coverage, and homogeneity. The concepts being distinct the nomenclature must be kept equally distinct. It is hardly necessary to cite specific examples of confusion of these concepts for it is everywhere in the literature. A species of high numerical index need not be also of high coverage or of high frequency. In an old field, for example, pennyroyal (*Hedeoma*) may be present in very large numbers and still, because of its small size, be of the lowest class of dominance. At the same time it may be of regular distribution throughout the community and consequently of high frequency, or irregularly distributed in patches and of low frequency. It has been a common error to speak of a species of high frequency as one of the "dominants" of a community, which may or may not be so. One plant only of a species in each quadrat examined would give a species a frequency of 100 per cent, the same as if it were of high density in some or all of the sample areas. It will be a dominant or not only according to its coverage in its stratum and not because of either frequency or density. Adamson (1931) states that "in many communities it is found that the species with the highest frequency indices are not those that would on other grounds be looked upon as most important in the community. . . . In other words the species that give the distinctive features to the community are not those most generally distributed throughout the area."

Expressions of abundance are more common in the literature than of density since the former is estimated and the latter depends upon actual counts within limited areas and entails considerable labor. The value of abundance estimates depends on the extent of area, richness and variability of the flora, and the ability and experience of the estimator. It is frequently impossible, or at least impractical, to replace abundance data by density figures because of the time and cost involved and the extent of the vegetation to be considered. It becomes increasingly important then that there be some means of standardizing abundance estimates and expression. The five classes of Braun-Blanquet and Pavillard have been cited. The fact that a limited number of classes is used for degrees of abundance is more important than the description of those classes. Tansley and Chipp (1926) suggests the following: (1) *r*, rare; (2) *o*, occasional; (3) *f*, frequent; (4) *a*, abundant; and (5) *va*, very abundant. Hanson and Love (1930b) also use a five point scale: (1) *vs*, very scarce; (2) *s*, scarce; (3) *i*, infrequent; (4) *f*, frequent; and (5) *ab*, abundant. The objection here lies in the use of the word "frequent," which has another and technical meaning in phytosociology. A. W. Sampson (1919) uses

the terms "very scattered," "scattered," "moderately dense," and "dense" and remarks that "abundance as here used takes into account the size of the plant and its herbage production as well as the density in which it occurs." From such a system it is apparent that one must be already familiar with the vegetation described to appreciate or to use the system when coverage and number are not kept distinct. Sarvis (1920) presents plant lists in which the species are arranged in the order of abundance; "the order of the dominant species was determined by measurements from quadrat maps (coverage) and in the field. The order of primary and secondary species, other than grasses, was by count. The secondary species are listed in the order of their estimated abundance." Petrie (1929) and others use a ten class system which mixes up all the quantitative concepts: *r*, rare; *o*, occasional; *lf*, locally frequent; *f*, frequent; *a*, abundant; *la*, locally abundant; *cd*, co-dominant; *ld*, locally dominant; *sd*, sub-dominant; *d*, dominant. Gause (1930) following Drude, indicates abundance as follows: *soc.*, social; *cop.*, copious; *spar.*, sparse; *sol.*, solitary. As we shall see later on "sociability" is one of the qualitative concepts and should not be confused with numerical abundance *per se*. It may be reiterated, the use of a limited number of abundance classes, preferably five, without confusion of number with coverage, frequency, sociability or any other aspect of vegetational structure, is highly desirable in descriptive work. Density is a very simple concept, which is illustrated in Table I.

TABLE I—Density of species in the *Picea-Abies*-Association, Mt. Le Conte, Great Smoky Mountains, Tennessee. The figures are given in number per square meter on 25 meter quadrats

Species	Stratum	Number
<i>Abies fraseri</i>	tree ¹	14
<i>Pyrus americana</i>	"	1
<i>Picea rubra</i>	"	0.12
<i>Vaccinium erythrocarpum</i>	Shrub	0.2
<i>Rubus canadensis</i>	"	0.16
<i>Menziesia pilosa</i>	"	0.08
<i>Oxalis acetosella</i>	herb	210
<i>Clintonia borealis</i>	"	15
<i>Aster paniculata</i>	"	12
<i>Solidago glomerata</i>	"	4
<i>Aspidium spinulosum</i> var. <i>intermedium</i>	"	2
<i>Asplenium filix-femina</i>	"	1

¹ Including transgressives.

Lutz (1928) in considering the composition and structure of a typical Red Cedar-Gray Birch-Association gives density figures by height classes for woody plants on a .075 acre quadrat. Forestry data is usually expressed on an acre basis. (Table II)

TABLE II—*Density of woody species in a Red Cedar-Gray Birch-Association, New England (Lutz, 1928)*

Species	Individuals per acre			
	0-6 ft. high	6-15 ft. high	over 15 ft. high	Total
Gray birch.....	251	66	238	555
Red cedar.....	119	106	79	304
Sugar maple.....	40	26	13	79
Scarlet oak.....	13	13
Dogwood.....	66	66
Rhus copallina.....	1082	1346	2428
Juniperus communis.....	330	330
Crataegus sp?.....	26	26
Total.....	1927	1544	330	3801

As previously stated, dominance according to the Swiss-French school is expressed in five classes of coverage $1/20$, $1/5$, and three classes increasing by $1/4$, successively. Hult-Sernander suggests the following divisions: *Do. 1*, less than $1/16$ of the area; *Do. 2*, $1/16$ to $1/8$ of the area; *Do. 3*, $1/8$ to $1/4$; *Do. 4*, $1/4$ to $1/2$; *Do. 5*, $1/2$ to all of the area. The Lagerberg-Raunkiaer technique, as reported by Romell (1930) consists of comparison with fixed standards (*e.g.* four sectors of the analyzing circle divided on the ratio, $5:2:2:1$). Table III compares the systems on a percentage basis.

TABLE III—*Comparison of three sets of coverage classes. Figures are given as percentages*

Class	Braun-Blanquet	Lagerberg-Raunkiaer	Hult-Sernander
Do. 1.....	1-5	1-6.25	1-10
Do. 2.....	6-25	6.5-12.5	11-30
Do. 3.....	26-50	13-25	31-50
Do. 4.....	51-75	26-50	51-100
Do. 5.....	76-100	51-100

Since dominance must be considered separately for each stratum of the vegetation there is sometimes confusion and for different purposes there are different methods of consideration. In the first place the coverage percentages indicated for the species composing a community will, in several-layered communities, frequently total in excess of 100 per cent. Again percentages are sometimes expressed on a basis of the total area covered by vegetation rather than the total area of land surface. Allen (1926), using dominance in the true sense of area of coverage by species brings out the point that in many types of vegetation, as rock-vegetation, that on fell-fields, screes, etc., all species have a very low percentage of cover. He qualifies the term in such instances as "physiognomic dominance" where certain species provide the

characteristic facies for such situations. The following outline of the different aspects of dominance, as the writer sees the problem, may help to clarify the matter.

A—Physiologic Dominance—a result of competition of species with species.

a—In general ecology: coverage of foliage (shading) mostly in lower vegetational strata.

b—In forestry:

1—Crown class, of the arborescent stratum,

2—Basal area, the cross-sectional area of trees from D. B. H.

c—In grazing: the basal area of the forage at one inch above the ground (as distinct from coverage in a).

B—Physiognomic Dominance—where all vegetation is widely spaced.

C—Life-form Dominance—where many species may be co-dominants, but are all comparable as to life-form.

Certain of the above concepts need some further discussion. In crown class the distinction of dominant, co-dominant, intermediate and suppressed involves both volume and height as well as density of shading from the foliage. Also sample plots are frequently mapped on the basis of the crown spread of each tree, in which case crown dominance can be expressed in terms of area for each species.

Basal area of the foresters is usually expressed as square feet per acre for each species. The diameter breast high (D. B. H.) measured in inches at four and one-half feet above the ground is changed to cross-sectional area and added up for each species. Korstian and Stickel (1927) make good comparative use of this type of data in studying natural replacement of blight-killed chestnut (Table IV).

TABLE IV—*Density and basal area in a Chestnut-Oak Forest before and after blight ravages (Korstian and Stickel, 1927)*

Species	1910-1911		1924	
	Trees per acre	Basal area per acre in sq. ft.	Trees per acre	Basal area per acre in sq. ft.
Chestnut.....	053.3	64.18	0	0
Red oak.....	22.0	9.13	38.0	16.45
Chestnut oak.....	22.0	5.25	52.7	14.48
White oak.....	9.4	1.97	9.4	2.07
Black oak.....	2.0	.82	3.3	.94
Scarlet oak.....	8.0	2.75	5.3	3.31
Sweet birch.....	.7	.13	0	0
Red maple.....	0	0	8.7	.82
White ash.....	0	0	6.7	.32
Hickory.....	0	0	6.0	.29
Sugar maple.....	0	0	2.7	.26
Total.....	217.4	84.23	132.8	38.94

In grazing work it was early discovered that the total spread of the foliage was practically useless information. At different times during the year and at the same date on different years the forage plants varied widely in their spread, or coverage in the ordinary sense. Also the extent and severity of grazing is of tremendous importance in varying the spread of the foliage, as well as the time interval since the last cropping. Consequently a concept of basal area was developed and forage plants are mapped according to their coverage at one inch above the ground, which is considered the height of normal utilization. In Fig. 1 the inner circles indicate basal area which gives

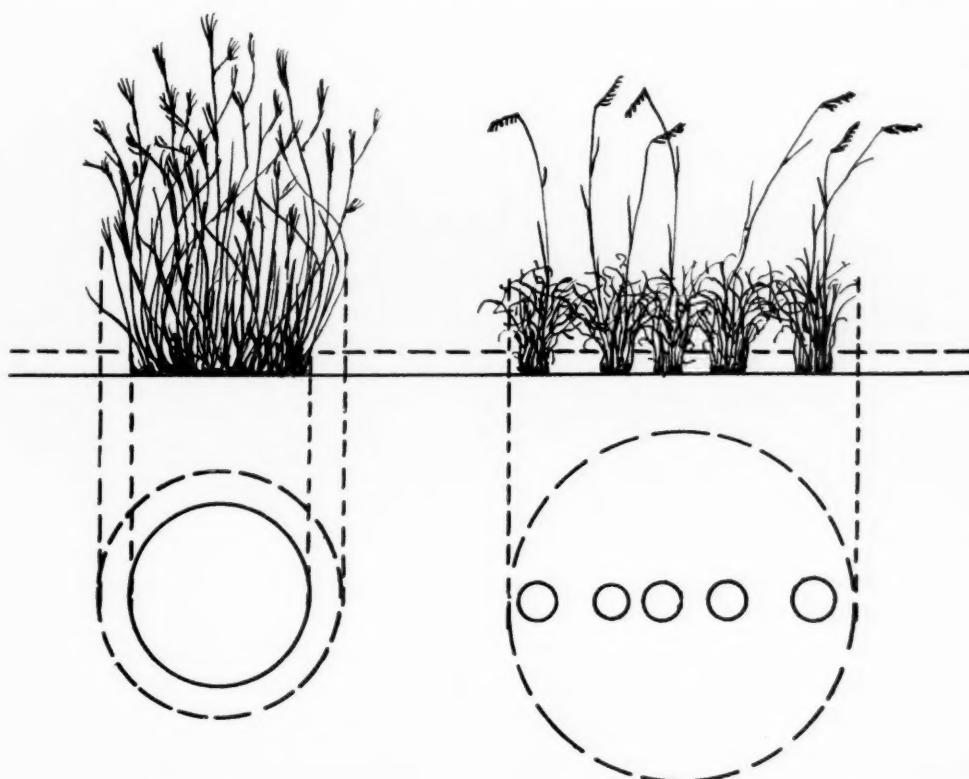


FIG. 1. *Aristida purpurea* (on the left) and *Bouteloua gracilis* to show the difference between maximum spread of the foliage and basal area. Basal area is the extent of coverage at one inch above the soil, which is approximately the height of maximum cropping. The error in mapping maximum spread may be very great since it is so strongly influenced by the season, time and severity of grazing, etc.

comparable and reliable data on different examinations of the same quadrats. The outer circles represent foliage cover at its maximum spread for the life-forms represented by two species. It is apparent that the error in comparison of foliage cover at different times could amount to several hundred per cent in some types, if not on a basis of basal area.

Adamson (1931) has recently pointed out that certain brush communities in South Africa which are subject to repeated fires exhibit "life-form dom-

inance." In various assories and to a certain extent in the climax no one species, or even a small number of species, exerts strict dominance, but collectively several species all of the same life-form dominate a plant community. These species have a high degree of interchangeability within any particular plant community. Adamson (1931) states "as compared with species dominance, life-form dominance leads to more varied communities from the floristic standpoint and to less definitely fixed populations. In succession the passage from one stage to another is not at all necessarily associated with a change in the dominant life-form. The change often occurs through regroupings of the important classes; and a series of stages, each distinct in itself, may have the same general distribution of life-forms. Again life-form dominance may at any stage pass into species dominance when one member of a class increases at the expense of the others." Life-form dominance, with its interchangeability of species is not only found in the communities of Table Mountain described by Adamson, but occurs in grasslands (Clements, 1928), is common in tropical forests, and may occur in one stratum and not in another of any community. It has been suggested that it is more characteristic of old floras and hence less common in the north temperate region.

This matter suggests a situation which the writer has observed. In hydrarch successions in the northeastern part of the United States and adjacent Canada there is frequently a conifer stage which is later supplanted by a deciduous swamp forest. This conifer stage may consist of tamarack (*Larix laricina*), white pine (*Pinus strobus*), white cedar (*Thuja occidentalis*), red spruce (*Picea rubra*), black spruce (*Picea mariana*), etc. Any one or more of these species may dominate the conifer stage. It is a matter of life-form dominance by narrow-sclerophyll trees when the community is considered in the abstract and may or may not be in any concrete example.

Frequency has probably received as much attention by American ecologists as all the rest of the concepts together. This may be due to the theoretical interest in Raunkiaer's so-called "Law of Frequency" which states that in an association at a state of *relative equilibrium* one or several species prosper at the expense of their neighbors and constitute the species to be found in frequency classes D and E (4 and 5), especially the latter, while many sporadic species of low frequency fill up class A. Incidentally Kenoyer (1927) speaks of class E species as a "considerable showing of dominants." It must be reiterated that the dominants in a plant community, i.e., species of high coverage, are usually also of high frequency, but commonly species of high frequency are of low coverage and hence not "dominants." Nichols (1930) suggests the term "distribution index" in the place of "frequency index" to avoid confusion with ideas relating to abundance. This is not necessary if the term "frequency" is not used to describe any of the five abundance classes of Braun-Blanquet and Pavillard.

Raunkiaer (Romell, 1930) on a basis of over eight thousand percentages found the following distribution through the five frequency classes: Class A, 53 per cent of the species; B, 14 per cent; C, 9; D, 8; and E, 16 per cent. Kenoyer (1927) working on American plant communities found the peak in Class A to be higher than for European communities as determined by Raunkiaer, while the second and lower peak in Class E was even lower than that of Raunkiaer. Kenoyer's classes, based on over fourteen hundred percentages, were as follows: Class A—69 per cent of the species, Class B—12, C—6, D—4, and E—9 per cent. If these relationships are expressed in a curve we have a graphic representation of the "Law of Frequency." Frey (1927) writing on the graphic in plant sociology calls attention to the fact that P. Jaccard was the first to use such curves to express frequency. Similar curves have been used to express homogeneity, presence, constance, etc. Frey (1927), Gleason (1929), Nichols (1930), and Romell (1925 and 1930a) have discussed the significance of this "Law of Frequency" and seem to conclude that it is merely an expression that there are more species with a few individuals than with many. Romell (1930a) shows that by ordinary statistical procedure with three simple and clear assumptions, theoretical curves will result which check very well with the empirical frequency percentage curves. His assumptions were: (1) every one of the species in the community is adapted to a definite site; (2) the site factors in an area or district analyzed vary from point to point according to pure chance around a mean value; (3) on the average it is just as probable that species will be adapted to the rarer combinations of site factors as to those common in the particular area.

The size of the quadrat to be used in the investigation of a plant community is one which should be given careful consideration. Many sizes of sample plots have been used. Raunkiaer used the following areas: 0.01 sq. m., 0.1 sq. m., 1 sq. m., 10 sq. m. for different types of vegetation from the soil layer to the superior arborescent stratum. Pound and Clements (1898) used areas of 25 sq. m. Gleason (1920) used areas 1 sq. m. and 4 sq. m. When only woody species were considered Kenoyer (1927) used areas of 50 sq. m. and 100 sq. m. Du Rietz (1930) uses a sample area of 4 x 4 m. for forest investigations. Romell favors 0.1 sq. m. largely used by Raunkiaer. Hanson and Love (1930a) in a fine investigation found an area 1 x 2 m. best for certain grazing problems. Most common of all, however, has been the use of 1 m. quadrats. According to C. F. Korstian, in private communication, "foresters commonly use the milacre (6.6 feet square) in quadrat work. It contains .001 acre. Multiples of the milacre, 3.3 feet squares and 3.3 x 6.6 feet rectangles are also commonly used, especially in recent years."

Gleason (1929) shows that "the frequency of a species, as the term is ordinarily understood, is shown only when the quadrats are of such a size that the resulting indices cover the whole range of classes from one to five.

and when of this size the double peak in classes A and E is often apparent." Braun-Blanquet (1928) shows that the size of the quadrat used for the analysis of a piece of vegetation is determined at its lower limits by the minimum-area; *i.e.*, the size which in increased results in little if any change in the number of species encountered. Hanson and Love (1930a) on the matter of quadrat size in grazing studies conclude from three statistical methods of analysis of their data that the minimum area was one, or better, two square meters. The increased cost of larger sizes precluded their use since there were no commensurate advantages.

Arrhenius (1921) studied the relation between the size of the sample area and the number of species it would be expected to contain and arrived at the following formula:

$$\frac{\text{Area 1}}{\text{Area 2}} = \frac{(\text{Species 1})^n}{(\text{Species 2})}$$

Although the straight line relationship exists for the smaller sized quadrat it very soon fails as Gleason (1928) has pointed out, so that further increase in

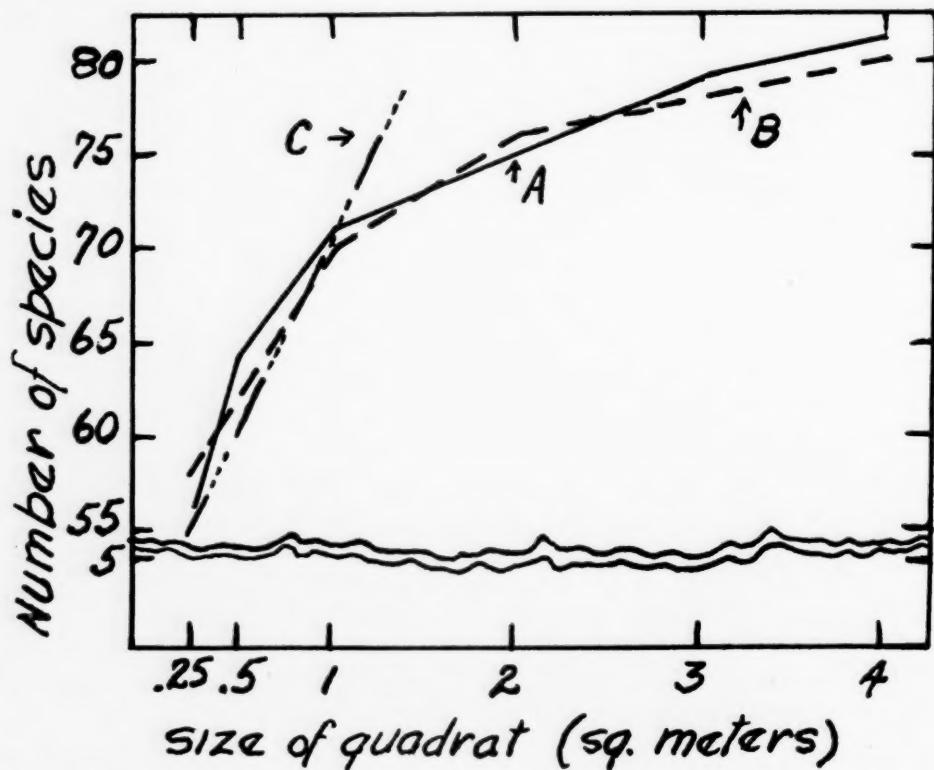


FIG. 2. The number of species in a total of 30 quadrats according to the size of the quadrat, after Hanson and Love. A, continuous line, the deferred and rotation pasture; B, broken line, the continuously grazed pasture. C, dot and dash line, represents Arrhenius' straight-line relationship, the number of species varying directly with the size of the quadrat. (Except for the addition of C this figure is copied from the above authors.)

area results in relatively slight increase in the number of species, the relationship being more nearly logarithmic than arithmetical. Kenoyer, in an as yet unpublished manuscript which he kindly allowed me to read, has discussed the logarithmic nature of the curves in detail. Data from various sources gave a straight line when plotted on logarithmic or semi-logarithmic paper (Fig. 2). Romell (1925) has compared the form of F-% curves, which are attendant on four very simple assumptions, with those of empirical origin. The fourth assumption, "that the material of the species is distributed over the different classes of frequency as they would be over the classes of elementary probability of 1,000 cases, ecologically different, which result in the free combination by chance of three ecological factors varying fortuitously, if one distinguishes for each factor ten classes of intensity," is the only relation theoretically deduced which agrees approximately with experience and approaches reality. This is a relation approximately linear between the logarithm of the area and the number of species.

In respect to frequency, as Gleason has pointed out, any decrease in size of the quadrats tends to move the index of the species into progressively lower classes of frequency and may completely remove the peak in Class E. Any increase in size of the sample areas tends to move them into higher classes, producing or augmenting a peak in Class E. Presumably a curve somewhat like Raunkiaer's or Kenoyer's (1927) "standard" curves would be a check on the adequacy of the sampling method (number and size of the plots) in any particular investigation. Hanson and Love (1930a) did not find this to be the case, but the explanation may rest in the fact that their vegetation was not undisturbed. Figure 3 gives the "Standard" curves of Raunkiaer and Kenoyer, and in addition other suppositional curves to show the effect of increase or decrease of quadrat size, the theoretical limit to this procedure being straight vertical lines in Classes A and B. One hundred per cent of the species would tend to be in Class A when the quadrats were so small as to include only one plant each. One hundred per cent would tend to be in Class E when the quadrats were so large as to include all species of the community in each sample area. This tendency, however, would be somewhat counterbalanced by the fact that larger quadrats would include some additional "rare" species (Fig. 3).

Romell (1930) demonstrates that it is not generally possible to translate frequency numbers into density. It is also apparent from the foregoing discussion that statistics from different sizes of sample areas can not be directly compared, although several investigators, even recently, use various sized sample areas in the same analysis. It therefore becomes highly desirable to arrive at a general agreement as to the standard sizes of sample areas. The scale of sizes in Table V is suggested for different purposes.

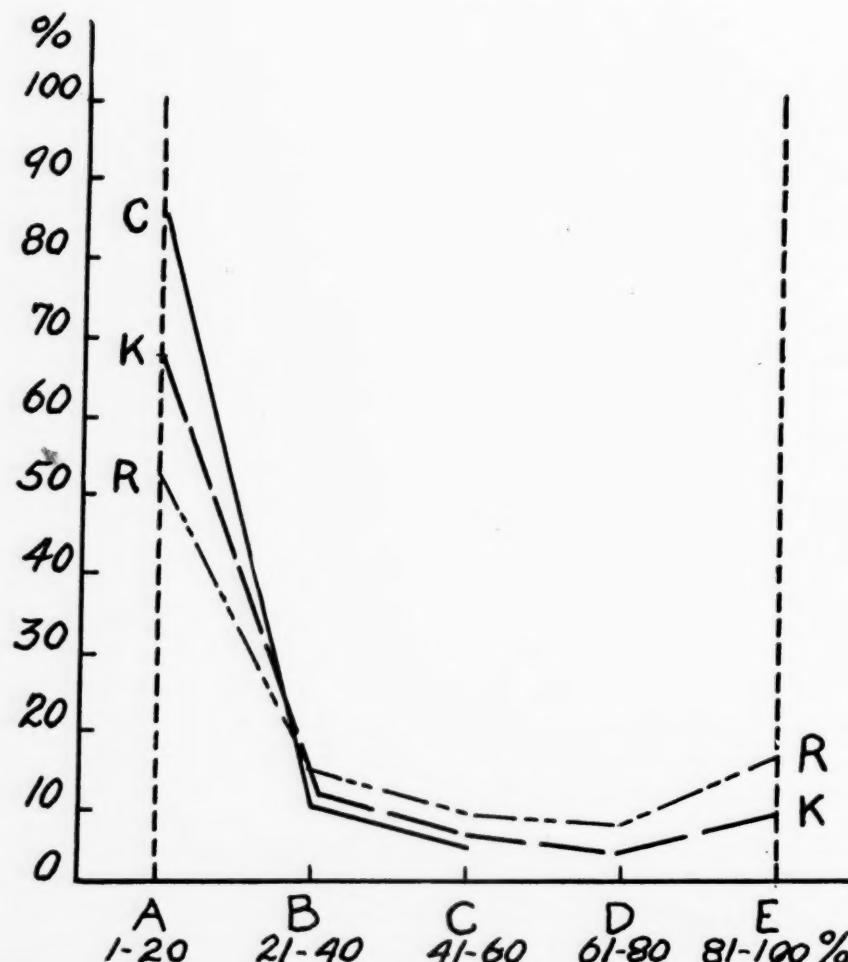


FIG. 3. Raunkiaer's (R) and Kenoyer's (K) standard curves of frequency. C is a study by Cain in which the quadrat was too small. Class D and E are absent while A is inordinately large. The vertical dotted lines at A and E represent the theoretical maxima with diminution and increase in the size of sample areas respectively.

TABLE V—Suggested Standard Quadrat Sizes

Standard sizes	Purpose, i. e. vegetational stratum	Approximate English equivalent in use in forestry, etc.
.01 sq. m.	Soil layer.....	
.1 sq. m.	Soil layer.....	
1.0 sq. m.	Herbaceous layer.....	1 yd. (1 m. = 39.37 in.)
2.0 sq. m. (1 X 2)....	Herbaceous layer.....	
4.0 sq. m. (2 X 2)....	Herbaceous or shrubby layer.....	1 milacre ¹ (6.6 X 6.6 ft.) (2 m. = 6 ft. 6.74 in.)
16.0 sq. m. (4 X 4)....	Inferior arborescent layer.....	
100.0 sq. m. (10 X 10)....	Superior arborescent layer.....	25 milacres ($\frac{1}{2}$ chain or 33 ft. square) (10 m. = 32.8 ft.)

¹ The milacre is perhaps the most commonly used unit in forestry quadrat work.

It is probable that for most work the difference between a sample plot 10 x 10 m. or of 1/2 chain on a side would be negligible. And so, as shown in the discussion of the area-species relationship, it would make even less difference (in frequency) for larger sizes of plots, as one chain on a side or 20 x 20 m., etc.

In regard to the number of quadrats necessary in an investigation nothing absolute can be stated. Kenoyer (1927) found 25 quadrats of suitable size to be satisfactory in his studies of frequency. Adamson (1931) says in regard to his work, "In some communities 50 sample quadrats were made; of these 25 were scattered at random and 25 were arranged in straight lines equally spaced. The figures for each set of 25 were calculated separately as well as those for the whole fifty. Further, the sample quadrats were grouped in pairs giving 25 samples double the area. . . . An examination of the figures so obtained shows a good degree of consistency for one meter quadrats, but a decided alteration when the size is doubled. . . . The agreement that is shown from the findings from 25 and from 50 quadrats was considered sufficiently close to enable the smaller number to be regarded as sufficiently adequate for sampling. As the scattered quadrats contained a larger number of species this arrangement was generally followed."

III. QUALITATIVE ANALYTIC CONCEPTS

In the preceding section quantitative analytic concepts of the organization of a plant community have been considered. There are now to be considered four qualitative concepts that do not depend on quadrat studies for their most accurate discernment, but rather on as wide an experience as possible with the community. These aspects of a plant community are called sociability, vitality, periodicity, and stratification.

SOCIABILITY. Sociability concerns the manner in which the species are disposed with reference to each other, as individuals or groups, in the interior of a given plant community. The assignment of sociability classes to species is subject to considerable variation, the limits of the classes by their very nature being somewhat vague and the personal element entering so largely. However, the limitation of sociability assignments to five classes helps very materially in standardizing this data. After Braun-Blanquet and Pavillard (1928) we list five classes:

- Soc. 1—growing isolated,
- Soc. 2—growing in groups,
- Soc. 3—growing in numbers,
- Soc. 4—growing in little colonies, and
- Soc. 5—growing in large colonies.

It is immediately apparent that the descriptive phrases help only a little in learning the value of these classes,—simply to indicate a scale of increasing

gregariousness. The citing of species to the classes helps only if the sociability of the species is familiar to the reader, yet this, it seems, is the only way that the five categories will ultimately take on any communicable reality. So it is with some hesitation that the illustrations in Table VI are ventured by the writer, knowing that any of the species allocated to a certain class may occur elsewhere in higher or lower classes.

TABLE VI—*Species from various communities to illustrate sociability classes*

Soc.	Picea-Abies-Association Smoky Mts., Tennessee	Sphagnum-Vaccinium-Associes Bogs near Lake Michigan
1	<i>Viola blanda</i>	<i>Cardamine pratense</i>
2	<i>Saxifraga leucanthemifolia</i> <i>Parnassia asarifolia</i>	<i>Sarracenia purpurea</i> <i>Menyanthes trifoliata</i>
3	<i>Chelone lyoni</i> <i>Clintonia borealis</i>	<i>Eriophorum virginicum</i>
4	<i>Senecio rugelia</i> <i>Solidago glomerata</i>	<i>Andromeda glaucophylla</i> <i>Chamaedaphne calyculata</i>
5	<i>Oxalis acetosella</i> <i>Aspidium spinulosum</i> var. <i>intermedium</i>	<i>Sphagnum cymbalifolium</i> , etc. <i>Vaccinium palustre</i>
Soc.	Fagus-Acer-Association, Herbs, Indiana	Fagus-Acer-Association, Woody plants, Indiana
1	<i>Conopholis americana</i> <i>Botrychium obliquum</i> <i>Hexalactis aphylla</i>	<i>Nyssa sylvatica</i> <i>Juglans nigra</i>
2	<i>Pogonia trianthopora</i>	<i>Tilia glabra</i> <i>Juglans cinerea</i>
3	<i>Epifagus virginiana</i> <i>Trillium sessile</i>	<i>Prunus serotina</i>
4	<i>Podophyllum peltatum</i> <i>Asarum canadense</i>	<i>Asimina triloba</i> <i>Benzoin aestivale</i> <i>Dirca palustris</i>
5	<i>Claytonia virginica</i>	<i>Acer saccharum</i> <i>Fagus grandifolia</i>

VITALITY. Vitality concerns the degree of vigor and prosperity attained by the different species. Three vitality classes are usually recognized, with corresponding symbols or class numbers.

Vi. 1—(Symbol, o) plants germinating accidentally and not able to multiply.

Vi. 2—(Symbol, ⊙) plants with their life cycle incomplete but with vigorous vegetative development.

Vi. 3—(Symbol, ●) plants well-developed and accomplishing regularly their complete life cycle, flowering and fruiting.

It is frequently said that species at the margin of their geographic range are of low vitality but that is certainly not always the case, Griggs (1914). It is more likely to be true when a species is at its ecologic limits, which is not the same as the geographic limits since many species have not spread throughout their potential range. It has also been frequently observed that plants fruit heavily when under extenuating circumstances and relatively poor vegetative development.

PERIODICITY. Periodicity in a plant community refers to the different aspects of the community during the year. In many regions it is practicable to indicate the seasonal aspect:

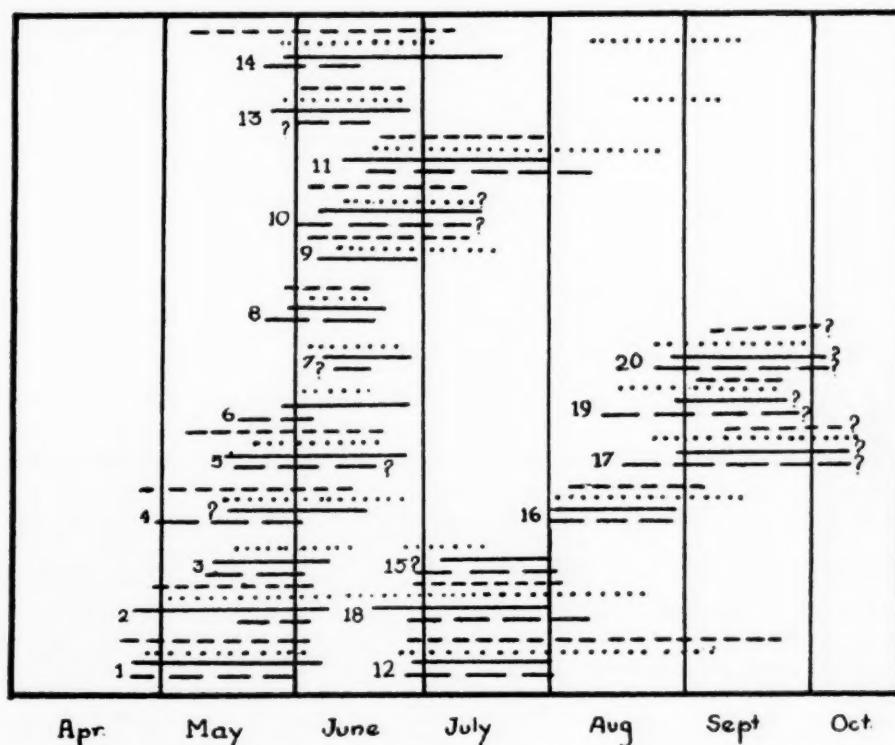


FIG. 4. From Hanson and Love. This illustrates a common method of presenting phenological data. Here the length of the line represents the length of the flowering period.

Pc. 1, vernal; *Pc.* 2, aestival; *Pc.* 3, autumnal; *Pc.* 4, hibernal.
Periodicity can also refer to the ontogeny of the species as Braun-Blanquet (1928) suggests:

fol.—leafy period (Pflanze beblattert)

s.fol.—leafless period (Entblattert)

fl.—flowering period (Blühend)

fr.—fruiting period (Fruchtend)

kl.—embryo period (Keimling)

ass.—assimilating period (Assimilierend)

The preceding systems can be combined in the following manner:

Dicentra canadensis—fol. 1, fl. 1

Podophyllum peltatum—fol. 1-2, fl. 1

Hamamelis virginiana—fol. 1-3, fl. 3-4.

Rhododendron maximum—fol. 1-4, fl. 1-2

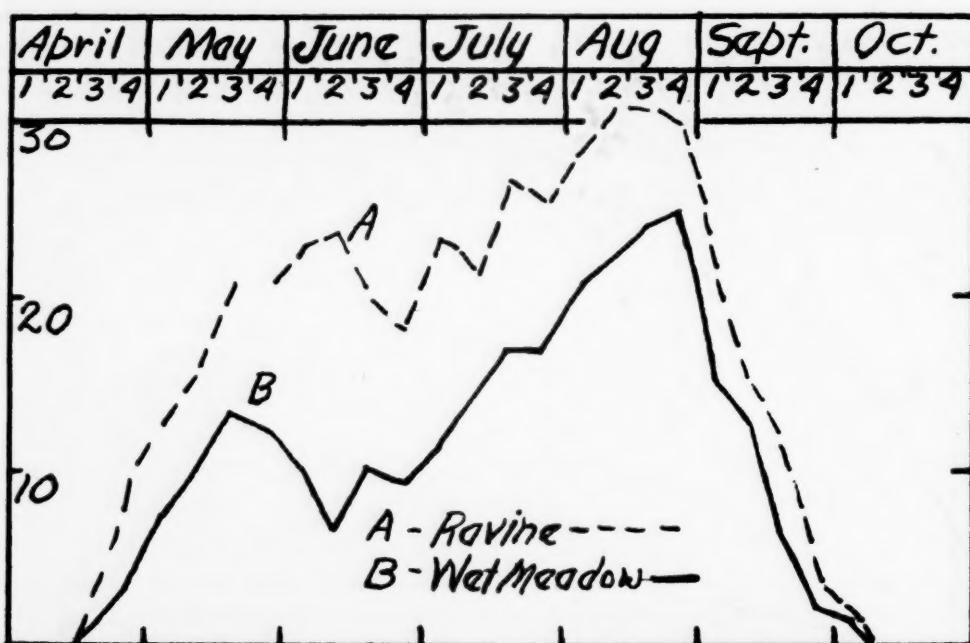


FIG. 5. Graphs showing the number of species flowering at different times during the growing season; A, in ravine and B, in wet meadow. After Steiger (1930).

The flowering period of plants, which has been most studied from the point of view of periodicity, is usually diagramed by a line for each species which represents by its length the period and duration of flowering (Fig. 4) (Hanson and Love, 1931). Some authors (Steiger, 1930) show the peaks in the flowering season by diagraming the total number of species in flower at each week throughout the whole season (Fig. 5).

STRATIFICATION. Stratification refers to the natural superimposed layers of the vegetation. The number of strata distinguishable vary in different types of vegetation. In forests four strata can usually be distinguished.

St. 1—moss-lichen stratum,

St. 2—herbaceous stratum,

St. 3—shrubby stratum, and

St. 4—tree stratum.

It is frequently possible to discern superior and inferior layers in these strata. Low-creeping plants as *Mitchella repens*, *Epigaea repens*, *Linnaea borealis*, etc., present a difficult problem, but are not assignable to the moss-lichen layer or soil layer. Immature plants, like tree saplings in the shrub layer,

are referred to as "transgressives" since they are "passing through" the lower stratum and will ultimately occupy the higher arborescent stratum. Transgressives of this sort if of high abundance are of special interest in indicating possible future dominants in the superior stratum (Cain, 1932; Lutz, 1928). A very interesting and instructive diagram (Fig. 6) combines stratification and dominance data; see Braun-Blanquet (1928) and Lindsey (1932).

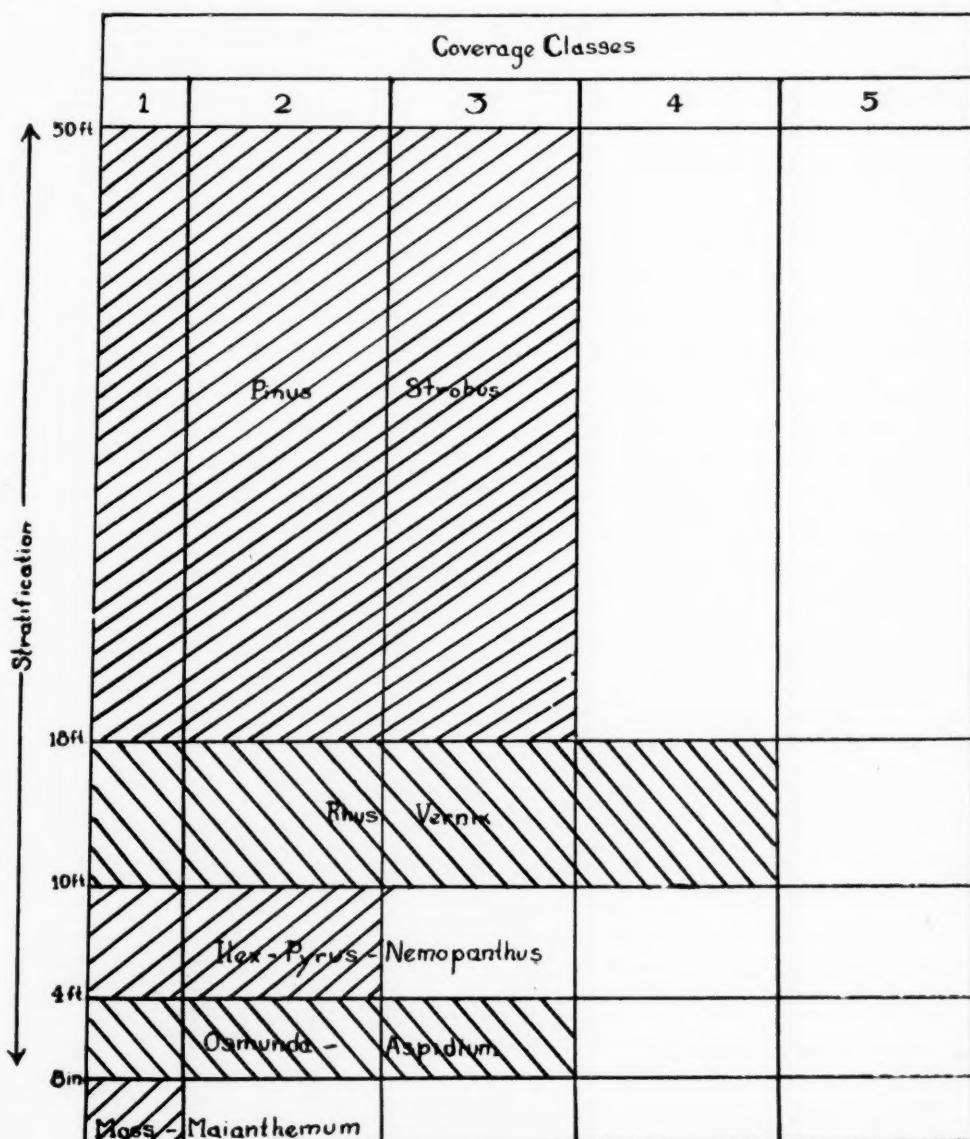


FIG. 6. A combination of coverage class data and stratification. This represents a pure white pine bog forest with two understories of shrubs and one of ferns and moss. This sort of diagram is of value in forestry to indicate the condition of the reproduction of various ages, the feasibility of clear-cutting, etc. After Lindsey.

Stratification of subterranean organs is probably as important as that of shoots but it is much more difficult to study and is usually ignored. Weaver

(1919) is probably foremost in developing such considerations. Recent work by Toumey (1929) and later carried on by Toumey and Kienholz (1931) shows the importance of root-competition.

IV. SYNTHETIC CONCEPTS REFERRING TO THE ORGANIZATION OF A PLANT COMMUNITY IN THE ABSTRACT

The concepts so far have dealt with any one concrete piece of vegetation of distinct kind. Most ecologists have accepted the use of the term "association" for example, in both its concrete use when referring to some particular stand and also in the abstract sense when referring to the synthetic picture of a plant community which is constructed from as many of its concrete examples as can be studied. It is comparable to the use of the term "man" in reference to one individual on one hand and to mankind on the other. Or, as Nichols (1929) points out, "By some ecologists, the term association is applied to the concrete piece of vegetation which we study in the field, and which corresponds to the individual plants of the taxonomist. By others, the individual pieces of vegetation are regarded merely as examples of an association, in much the same way that different individual plants of the same kind may be regarded as examples of a particular species." The fundamental unit of plant sociology is the plant association. Disregarding, for the present purpose, Clement's admirable distinctions of consociation, and the seral communities, associates, consocies, etc., and using Nichol's (1929) definition, "we may characterize the plant association as a piece of vegetation which exhibits essential uniformity in two respects, namely, floristic composition and ecologic structure. The term 'ecologic structure' is one that I have used to include all features of a plant community which are of ecological importance. The physiognomy of a plant community, for example, is but one expression of its ecologic structure. . . . My point of view, in brief, would be simply this: (1) that floristic uniformity may be significant in indicating uniformity or lack of uniformity in the ecological structure of vegetation, (2) that, in so far as this is true, or even in so far as there seems a possibility that it may be true, so far, at any rate, it is imperative that floristics should be taken into account by the ecologist." With this in mind we are able to study and give tabular presentation to two aspects of the abstract association (or other group) namely, presence, or constance, and fidelity.

PRESENCE. Presence concerns the degree of regularity with which species reoccur in different examples of an association. No plant community enjoys a continuous distribution throughout the geographic range of the community, for climatic and especially edaphic factors are not so homogeneously integrated over wide territory, but is rather to be found here and there where the combination of factors is right. For example, the Acer-Fagus-Association as an abstract concept is to be known on a basis of concrete examples of the

association in Indiana, Michigan, New York, Tennessee, etc. If floristic lists are prepared of stands of the beech-maple association in different localities the presence of a species is a matter of the regularity with which it appears in those different, although essentially similar, floristic lists. Five classes are indicated.

Pr. 1—species found in 1-20 per cent of the concrete examples of the association studied.

Pr. 2—species found in 21-40 per cent,

Pr. 3—species found in 41-60 per cent,

Pr. 4—species found in 61-80 per cent, and

Pr. 5—species found in 81-100 per cent.

An illustration of the use of this concept is shown in Table VII.

TABLE VII—*The presence of thirty-four species of woody plants in seventeen stands of the Heath Bald Association (Cain, 1930)*

WOODY SPECIES	APPROXIMATE ALTITUDE										Presence Per cent							
	6,500 Feet					5,000 Feet						4,000 Feet						
	1	2	3	4	5	6	1	2	3	4	5	1	2	3	4	5		
Rhododendron catawbiense	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	100		
Vaccinium corymbosum	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	100		
Rhododendron punctatum	.	x	x	x	x	x	x	x	x	x	x	-	-	-	-	70		
Dendrium prostratum	.	x	-	x	-	x	x	x	x	x	x	-	-	-	-	60		
Vaccinium erythrocarpum	.	x	x	x	x	x	-	x	-	x	x	-	-	-	-	53		
Menziesia pilosa	.	x	x	-	-	x	-	-	-	-	-	-	-	-	-	23		
Pyrus americana	.	x	x	x	-	-	-	-	-	-	-	-	-	-	-	23		
Prunus pennsylvanica	.	x	x	-	-	x	-	-	-	-	-	-	-	-	-	18		
Picea rubra	.	x	-	-	x	x	-	-	-	-	-	-	-	-	-	23		
Abies fraseri	.	x	-	-	x	x	-	-	-	-	-	-	-	-	-	23		
Ribes prostratum	.	-	-	x	-	-	-	-	-	-	-	-	-	-	-	6		
Rubus canadensis	.	-	x	x	-	-	-	x	-	x	x	-	-	-	x	35		
Diervilla sessilifolia	.	-	-	x	-	-	-	x	-	x	-	-	-	-	-	18		
Kalmia latifolia	.	-	-	-	-	-	x	x	x	x	x	x	x	x	x	65		
Pyrus melanocarpa	.	-	-	-	-	x	-	x	-	x	x	x	-	-	x	41		
Lyonia ligustrina	.	-	-	-	-	-	x	-	x	-	-	-	-	-	x	18		
Gaylussacia baccata	.	-	-	-	-	-	x	-	-	-	-	-	-	-	x	12		
Nyssa sylvatica	.	-	-	-	-	-	x	-	-	-	-	-	-	-	x	12		
Ilex monticola	.	-	-	-	-	-	-	x	x	x	x	x	x	x	x	53		
Clethra acuminata	.	-	-	-	-	-	x	-	x	x	x	x	x	x	x	53		
Viburnum cassinoides	.	-	-	-	-	-	x	-	x	x	-	x	-	x	x	41		
Smilax rotundifolia	.	-	-	-	-	-	x	-	-	-	-	x	x	x	x	35		
Acer rubrum	.	-	-	-	-	-	x	-	x	-	-	x	x	x	x	41		
Robinia pseudo-acacia	.	-	-	-	-	-	-	x	-	-	-	-	-	-	x	12		
Andromeda floribunda	.	-	-	-	-	-	-	-	x	x	-	-	-	x	x	23		
Amelanchier canadensis	.	-	-	-	-	-	x	-	x	-	-	x	x	x	x	35		
Rhododendron maximum	.	-	-	-	-	-	x	-	-	-	-	x	x	x	x	35		
Leucothoe catesbeiana	.	-	-	-	-	-	x	-	-	-	-	x	x	-	-	18		
Oxydendrum arboreum	.	-	-	-	-	-	x	-	-	-	-	-	x	x	-	18		
Pinus pungens	.	-	-	-	-	-	-	-	-	x	-	x	x	-	x	23		
Sassafras variifolium	.	-	-	-	-	-	-	-	-	-	-	x	-	-	x	12		
Gaylussacia ursina	.	-	-	-	-	-	-	-	-	-	-	-	-	-	x	6		
Castanea dentata	.	-	-	-	-	-	-	-	-	-	-	-	-	-	x	6		
Hamamelis virginiana	.	-	-	-	-	-	-	-	-	-	-	-	-	-	x	6		
Number of species in each bald	.	10	8	10	8	7	9	16	7	12	10	12	11	10	14	12	14	22
Average number of species	.			9						11					14			

Floristic lists, woody plants only, are presented for seventeen different heath bald communities in the vicinity of Mt. LeConte of the Great Smoky Mountains of North Carolina and Tennessee. These communities are here grouped by altitude and numbered in each group. The different heath bald associations, in the concrete, have from 7 to 22 species while the heath bald association in the abstract is found to be composed of 34 species of woody plants. Two species, *Rhododendron catawbiense* and *Vaccinium corymbosum*, have a presence of 5. Two other species also belong to a high presence class. *Rhododendron punctatum* and *Kalmia latifolia* have a presence of 4. The number of species in each presence class is as follows: Pr. 1, 13; Pr. 2, 10; Pr. 3, 7; Pr. 4, 2; and Pr. 5, 2. These figures produce a curve suggestive of many derived from frequency studies on the association in the concrete.

CONSTANCE. Constance is an expression relative to the presence of species in different examples of an association. Constance differs from presence in that it is based on the species occurring in a unit area in each association rather than in the entire extent of each association. The smaller area examined should be sufficiently large, however, to give a representative list. Constance gives results more nearly comparable than presence since it limits the variability due to differences in the area of different stands. In presence the larger stands would be expected to have more species and especially enlarge the lower classes of presence. Although the straight line relationship between species and area of Arrhenius (1921) is not applicable to larger areas as explained by Gleason (1928) and Romell (1925) and others and discussed elsewhere in this paper, constance is nevertheless the more accurate concept for quantitative comparisons. Value remains, of course, in complete floristic lists for the total area of each stand in building up the concept of the association in the abstract. Some difficulty is encountered in attempting to make a complete list for an association in the concrete since the margins of the association are not always sharp and species from contiguous associations in the transition are apt to be included and thus lessen the picture of the individuality of the association. In constance where a limited area from the center of the stand is considered this difficulty is obviated. The similarity of the concept of constance to that of frequency is apparent.

"Constants" are usually considered to be the species which are represented in 90 per cent or more of the sample areas if ten classes are recognized, or in more than 80 per cent if only five classes are recognized. The number of constants depends on the number and size of the sample areas, Romell (1925), when the association is essentially homogeneous. In natural associations the number of constants increases at first rapidly with increased size of the sample areas, but soon becomes practically constant or of very slight increase. In fact the constancy curves, both for distribution of the species through the

classes and the number of species represented on a sample area basis, correspond remarkably to the F-% curves as to type.

The difficulties in both frequency and constance investigations are intimately bound up with the problem of homogeneity. In an homogeneous community the material of the component species may be distributed quite uniformly as individuals, but they can also, and more commonly, be found in groups, each including a large number of individuals separated by spaces where there are not any members of that species. If that mosaic character becomes pronounced enough one speaks of a complex of associations. Osvald has given a definition of homogeneity which is here repeated with the modification suggested by Romell (1925), "homogeneity consists of a *repetition of variations*, sufficiently frequent on a given surface, in the composition of the plant cover."

FIDELITY. Fidelity is a measure of the extent to which a species occurs exclusively in a single kind of plant community, in fact this is termed "exclusiveness" by Tansley and Chipp (1926). Five classes of fidelity are recognized by Braun-Blanquet and Pavillard.

Fi. 1—strangers, species appearing in a plant community accidentally.

Fi. 2—indifferents, species growing more or less abundantly in several groups,

Fi. 3—preferants, species existing more or less abundantly in several groups, preferring however, a definite group,

Fi. 4—electives, species found especially in one group but met with, although rarely, in other groups, and

Fi. 5—exclusives, species related almost exclusively to a definite group.

Species of fidelity from 3 to 5 are considered the "characteristic species" of the plant community. Species of highest fidelity are frequently those of the strongest ecological "preferences" or perhaps, to say it another way, of the narrowest ecological "tolerance"; or they may be species characteristic of extreme habitats of xerophytism, hydrophytism, salinity, etc. The "mixed nature" of communities floristically may be an expression of their lack of stability, which may later be attained as succession progresses, or it may be a matter of the optimal condition of the majority of the habitat factors for the majority of the species, so that competition among species is exceedingly severe. This point is adequately discussed by Lundegårdh in the recent English edition of his text "Environment and Plant Development" pages 281-284 under the heading, the biological law of relative effects.

Gleason (1926) in commenting on the ordinary concept of the plant association says that "species of plants usually associated by ecologists with a particular plant community are frequently found within many other types of vegetation." This lack of fidelity does not invalidate the association concept for, as Nichols (1929) in his criticism of Gleason's individualistic concept of

the plant association says, "It should be borne in mind that in attempting to classify the facts of nature, we are dealing with *merging phenomena*." Fidelity data by Cain (1930) shows the relationship between the species of the heath bald association of the Great Smoky Mountains and four adjacent forest types. This does not attempt the assignment of fidelity classes, however, on



FIG. 7. Graphic presentation of fidelity data after Sampson. The thickness of the lines represents the relative importance of the species.

a basis of the Braun-Blanquet system. So far as the writer knows this type of investigation is comparatively strange to American ecology. H. C. Sampson (1930) on the mixed mesophytic forest of northeastern Ohio uses an admirable type of table and diagram of fidelity (Fig. 7).

The Swedish ecologists consider the species of high constancy for the association as a whole and for its separate variants especially important in recognizing and classifying plant communities. The Swiss-French ecologists consider exclusiveness, species of high fidelity, as even of more importance as a diagnostic criterion of the plant community than constancy.

V. TERMS RELATING TO SYNECOLOGY

In their vocabulary Braun-Blanquet and Pavillard (1928) include a consideration of the station (the normal habitat of any particular community), life-forms and syncytial units (synusia, formations, etc.). Since the former and the latter concepts can not be applied to individual species of a community they are omitted from the present consideration. In addition to the life-form classes of Raunkiaer (Smith, 1913) (Fuller and Bakke 1918) the writer has included the leaf-size classes, also of Raunkiaer, since they equally submit to tabular presentation.

LIFE-FORM CLASSES. The recognition of life-form classes is elementary in all botanical knowledge. The difficulty has been in the delimitation of the classes on perfectly natural bases without the formation of too many categories and refinements. Largely through the efforts of Smith (1913) and Fuller and Bakke (1918) and McDougall (1931) in calling attention to Raunkiaer's work which was so generally unavailable there is a fairly wide familiarity with these concepts of the great Danish botanist. The biological spectrum has especially attracted attention and found various types of utility. The biological spectrum is an expression on a percentage basis of the number of species in a region in each of the life-form classes. It has been used comparatively to show the progressive effect on the life-form composition of the vegetation of a region with increasing latitude, Summerhayes and Elton (1928), increasing altitude Smith (1913), decreasing precipitation Paulson (1915) while probably its widest usage has been in contrasting floras of different regions, as in the work of Ennis (1930) on Connecticut and Taylor (1918) on New York, Long Island, etc. Raunkiaer's "normal" forms an arbitrary baseline from which differences in class percentages are apparent.

Following is a list of Raunkiaer's Classes with some of their subdivisions. The single theme of this classification, which is one of its main recommendation, is that of decreasing protection afforded the perennating bud from class IV to IX at least. Classes I to III are largely protected by spores. Class X may be of any degree of exposure as indicated by the subdivision of Class IX. An anomalous group entitled succulents is sometimes included.

Class I—Phytoplankton (Pp); floating microscopical organisms.

Class II—Phytoedaphon (Pe); microscopical organisms in the soil.

Class III—Endophytes (End.) inferior organisms living under the surface of rocks or in the tissues (intercellular-spaces) of plants or living animals.

Class IV—Therophytes (Th); annual plants with perennating bud which winters over in the seed.

Class V—Hydrophytes (HH); phanerogams with reproductive organs under water, *i.e.* perennating bud submersed.

A—Helophytes (HH); marsh plants with perennating bud at the bottom of the water or in the muck and soil beneath the water.

Class VI—Geophytes (G); phanerogams with vegetative reproductive organs in the soil, *e.g.* bulbs, rhizomes, tubers on stems and roots, root buds, etc.

Classes V and VI are collectively known as cryptophytes (C).

Class VII—Hemicryptophytes (H); phanerogams with vegetative buds at the level of the ground or the substratum. The aerial parts are herbaceous or semi-woody, and die away in the critical period. Some protection to the bud is afforded by the litter.

Class VIII—Chamaephytes (Ch); phanerogams with the perennating bud not over 25-30 cm. above the surface of the ground.

Class IX—Phanerophytes (P); phanerogams with the vegetative organs higher than 25-30 cm. above the ground, *i.e.* trees and shrubs (vines).

A—Nanophanerophytes (N), 1/4 to 2 meters high.

B—Microphanerophytes (M), 2 to 8 meters high.

C—Mesophanerophytes (MM), 8 to 30 meters high.

D—Megaphanerophytes (MM), over 30 meters high.

Class X—Epiphytes (E), vascular plants living on (attached to) other plants.

The normal biological spectrum of Raunkiaer is to be regarded as a good working hypothesis. The 400 species selected to compose it were carefully controlled in various ways and its significance lies in its use as a base-line. The outstanding features of other spectra are deduced by comparison of their amount of deviation from this arbitrary standard or phytoclimatic *rélevé*. Tables VIII, IX, and X illustrate the value of the spectra since the life-forms of the plants of a flora must be considered a reflection and integration of the climatic conditions.

TABLE VIII.—Altitudinal distribution of life-forms; Puschlav, Switzerland (Smith, 1913)

Altitude	No. Species	Percentage in life-form classes									
		S	E	MM	M	N	Ch	H	G	HH	Th
Above 2850 meters.....	51	35	61	2	..	2
2550-2850 meters.....	199	25	67	4	..	4
2250-2550 meters.....	348	1	3	18	64	7	1	6
1900-2250 meters.....	492	1	3	13	68	8	1	6
1550-1900 meters.....	487	3	4	11	62	10	1	8
1200-1550 meters.....	449	2.5	2.5	4	7	60	9	1	14
850-1200 meters.....	604	..	0.2	2	3	5	5	55	9	2	19
Below 850 meters.....	447	..	0.2	3	4	3	6	55	8	1	21
Normal spectrum.....	400	1	3	6	17	20	9	27	3	1	13

TABLE IX—Life-form spectra for the plant zones of Spitsbergen (Summerhayes and Elton, 1928)

District	No. Species	S	E	MM	M	N	Ch	H	G	HH	Th
Hope Island.....	20	25	70	5
Barren zone (1).....	38	26	66	3	5	..
Dryas zone (2).....	69	34	56	6	4	..
Cassiope zone (3).....	91	27	60	10	2	1
Fjord zone (4).....	127	1.5	21	58	15	3	1.5
Bear Island.....	53	2.0	26	53	11	5	2
Normal spectrum.....	400	1	3	6	17	20	9	27	3	1	13

TABLE X—Life-form spectra for five desert regions in the United States—Paulsen (1915)

The district	Rainfall	No. species	P (N)	Ch	H	G	Th
Akron, Colorado (H. L. Shantz).....	47 cm.	79	..	19	58	8	15
Tooele Valley, Utah (Kearney, et. al.).....	40 cm.	116	2	23	46	3	14
Tucson, Arizona (V. M. Spalding).....	27 cm.	266	18	11	..	24	47
Salton Sink, Arizona (V. M. Spalding).....	20 cm.	81	33	6	..	14	47
Death Valley, Calif. (Raunkiaer-Coville).....	294	26	7	18	2	42
Normal Spectrum.....	400	43	9	27	3	13

In the Northern Hemisphere Raunkiaer (Smith, 1913) recognizes three principal regional climatic zones: (1) a tropical area with uniform high temperature, but with varying humidity, (2) an area of decreasing warmth northward, increasing seasonal contrast, but with adequate precipitation and (3) an area similar to 2 but with inadequate precipitation. These climatic series are limited and sub-divided by *biochores* or plant-climate boundaries. They can be roughly characterized as of phanerophytic, hemicryptophytic and chamaephytic climate, respectively. In the Arctic region for example the chamaephytic percentage becomes an important guide and significance is attached to the

biochores indicated by 10, 20 and 30 per cent of chamaephytes. They agree approximately with recognized isotherms. Again, the subtropical climate with winter rains is characterized by evergreen phanerophytes with unprotected buds; temperate regions are distinguished by their essentially hemicyclopedia character; deserts by therophytic climate, etc. Cooper (1922) found the broad-sclerophyll scrub (chaparral) communities to be dominantly nanophanerophytic, over 80 per cent. Cain (1930) on the heath bald areas of the Great Smoky Mountains found the flora largely woody with some 70 per cent in the nano- and microphanerophyte classes. By far the most extensive work on Raunkiaer's life-forms in the United States is that on the flora of Connecticut by Ennis (1930) and Taylor (1918) on New York. An interesting paper by Phillips-Withrow (1932) deals with the life-form classes of relic extraneous and other communities in the Cincinnati region in comparison with their distribution elsewhere.

RAUNKIAER'S LEAF-SIZE CLASSES. Variation in leaf-size is a phenomenon in all floras but there is also obvious general differences between floras with larger leaf sizes more abundant in more tropical regions. Leaf-size classes should be used in conjunction with life-form classes, especially in the phanerophytic class. Leaf-size is a conspicuous, significant and easily determined characteristic of species and should be included in the sort of tabulation here recommended. Raunkiaer established six arbitrary classes on a natural basis as near as it is possible. He says, according to the translation of Fuller and Bakke (1918), "in determining the limits of a single class I took a progressive series of dry leaves varying much in size and separated them tentatively into six classes. I placed them upon millimeter paper and carefully noted where the boundary between the six classes could be set. I also persuaded a number of botanists among my colleagues to consider the same problem. The result has caused me to set the upper limit of the lowest size class, leptophylls, at 25 sq. mm. Five consecutive multiplications by 9 give products that respectively cover all the material." These classes follow:

Class 1—Leptophyll, 25 sq. mm.

Class 2—Nanophyll, 9×25 sq. mm. equals 225 sq. mm.

Class 3—Microphyll, $9^2 \times 25$ sq. mm. equals 2,025 sq. mm.

Class 4—Mesophyll, $9^3 \times 25$ sq. mm. equals 18,225 sq. mm.

Class 5—Macrophyll, $9^4 \times 25$ sq. mm. equals 164,025 sq. mm.

Class 6—Megaphyll, larger than Class 5.

Since these figures mean little to the eye a diagram is included (Fig. 8).

It is necessary not only to assign the leaves to size classes but first to classify them according to their character; evergreen or deciduous, simple or compound, etc. This is indicated in Table XI from the paper on the heath bald communities (Cain, 1930).

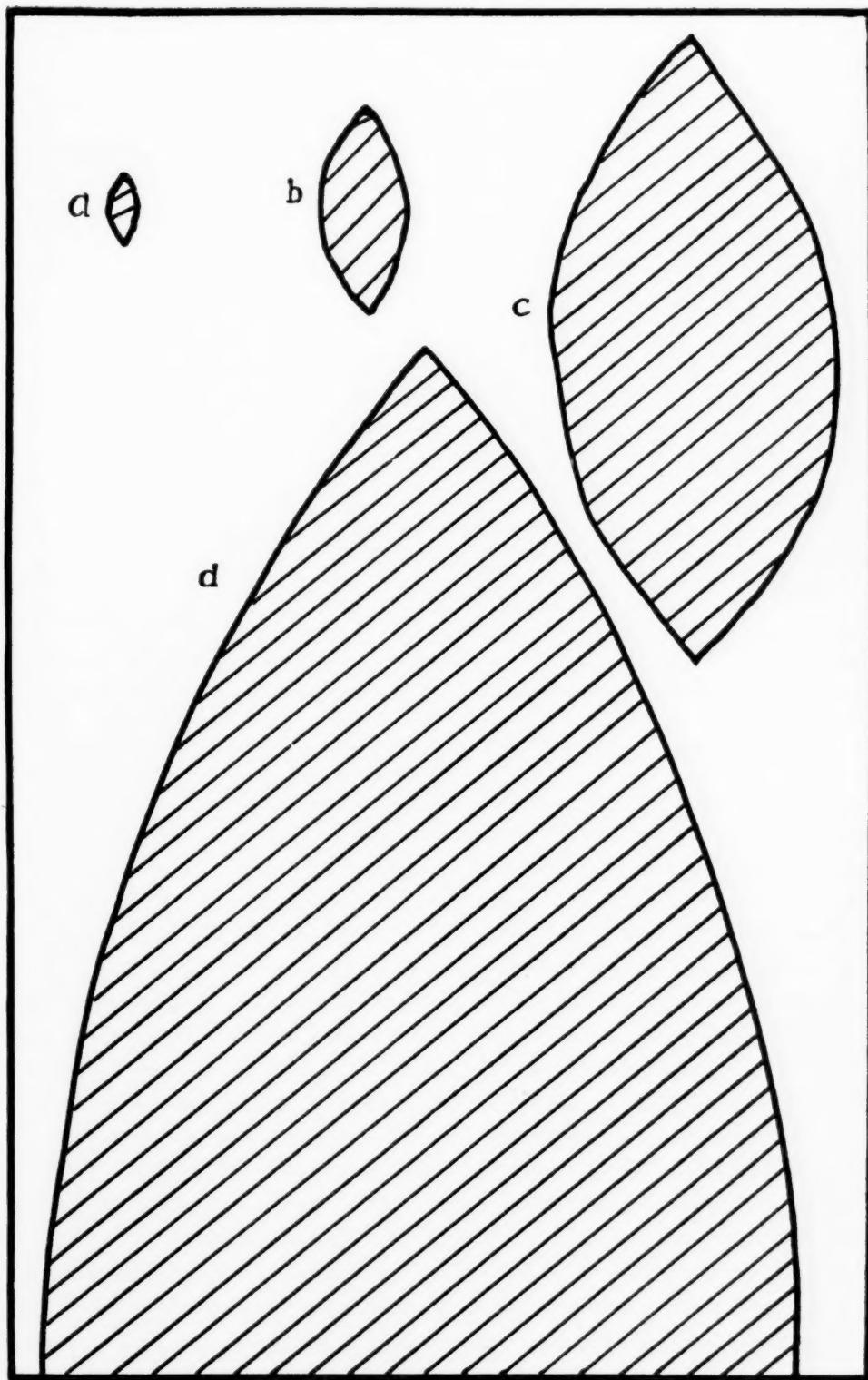


FIG. 8. Graphical representation of the limits between the different leaf-size classes. Leaves smaller than a are leptophylls; between a and b nanophylls; between b and c microphylls; between c and twice d mesophylls; between twice d and eight times the rectangle macrophylls and all larger leaves are megaphylls. After Fuller and Bakke.

TABLE XI—Leaf-size classification of the Heath Bald Flora of the Great Smoky Mountains, Tennessee (Cain, 1930)

Classification	Total flora		Woody flora		Ericales	
	No. sp.	%	No. sp.	%	No. sp.	Dominants
Aphyllous.....	1	2			1	
Evergreen.....	14	26	12	30	10	5
Scale-leaved.....	1
Needle-leaved.....	3	..	3
Broad-leaved.....	10	19	9	23	10	5
Leptophyll.....	1	..	1	..	1	1
Nanophyll.....	0
Microphyll.....	8	15	7	18	8	3
Mesophyll.....	1	..	1	..	1	1
Deciduous.....	39	72	28	70	10	1
Simple-leaved.....	31	57	24	60	10	1
Leptophyll.....	0
Nanophyll.....	3	..	2	..	2	..
Microphyll.....	22	41	17	42	6	1
Mesophyll.....	5	..	4	..	2	..
Macrophyll.....	1	..	1
Pinnately compound.....	8	15	4	10
Leptophyll.....	2
Nanophyll.....	3	..	1
Microphyll.....	3	..	3
Woody species.....	40	74	40	..	20	6
Herbaceous.....	14	26	1	..

VI. TERMS RELATING TO GENETICAL SOCIOLOGY

Braun-Blanquet and Pavillard (1928) consider four subjects under genetic sociology, *i.e.*, the genesis of plant communities or their natural evolutionary tendency towards the final climatic community or climax. The four subjects are: (1) dynamic behavior, which is the only one to be considered here since the others do not apply to individual species of the community, (2) succession, (3) synthetic units, and (4) syngenetic classification.

DYNAMIC BEHAVIOR. The dynamic behavior of a species concerns the influence of the species on the genesis of the communities of its sere. Five terms are employed to designate the classes of dynamic behavior:

- Class 1—those species that are constructive to the community,
- Class 2—those species that are conserving to the community,
- Class 3—those species that are consolidating to the community,
- Class 4—those species that are neutral to the community, and
- Class 5—those species that are destructive to the community.

The interpretation of these categories is sometimes rather difficult. The diagram in Figure 9 has been constructed in an attempt to show the rôles a species may play in seral communities. In this diagram Species 1 is *neutral*, since it extends equally throughout the stages of the sere. Species 2 is *destructive* to associates *a* since its appearance leads to associates *b* in which it is

constructive and also *consolidating* since it spreads out and becomes important quantitatively (*i.e.*, as to dominance, abundance, frequency). Species 3 plays the same rôle as 2 only for the associes designated by *b* and *c*. Species 4 is *conserving* since it is not a constituent of the succeeding associes.

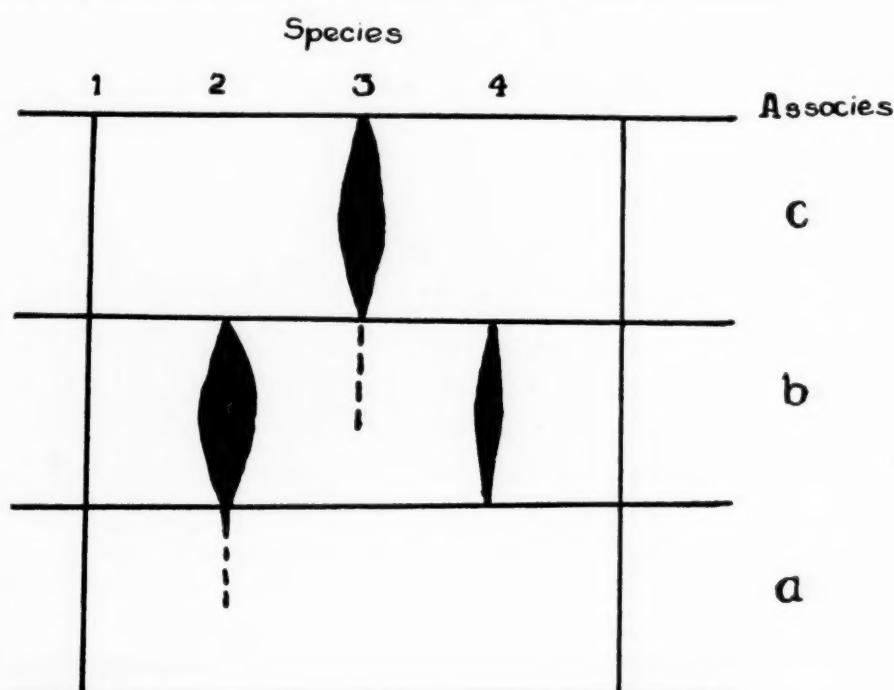


FIG. 9. A figure to illustrate the dynamic behavior of species. See text for details.

VII. SUMMARY

Plant sociological problems may be somewhat more difficult in America than in Europe, as some ecologists have suggested, but the writer is convinced that there are many ecological problems where the sort of study outlined in the present paper can be carried on without too great difficulties and with considerable advantage to the science. Many aspects of the work are familiar to American workers and have long been utilized to advantage, yet a complete study of even one community remains to be made. Figure 10 summarizes and shows the organization of the concepts which have been considered in this paper.

CONCEPTS RELATING TO THE SPECIES COMPOSING A PLANT COMMUNITY														
I. ORGANIZATION														
A. ANALYTIC					B.					II. SYN-ECOLOGY				
1. QUANTITATIVE					2. QUALITATIVE					SYNTHETIC				
a. ABUNDANCE	b. DENSITY	c. DOMINANCE	d. FREQUENCY	e. SOCIALITY	f. VITALITY	g. PERIODICITY	h. STRATIFICATION	i. PRESENCE	j. CONSTANCE	k. FIDELITY	l. LIFE - FORM	m. LEAF - SIZE	n. GENETIC	o. SOCIOLOGY
1-5	-	1-5	1-5	1-5	1-3	1-9	1-9	1-5	1-5	1-5	1-5	1-6	1-5	
SPECIES														
"	"	"	"	"	"	"	"	"	"	"	"	"	"	"

FIG. 10. A table for the presentation of phytosociological data concerning the species of a community in which it is possible to condense quantities of data by use of appropriate symbols discussed in this paper.

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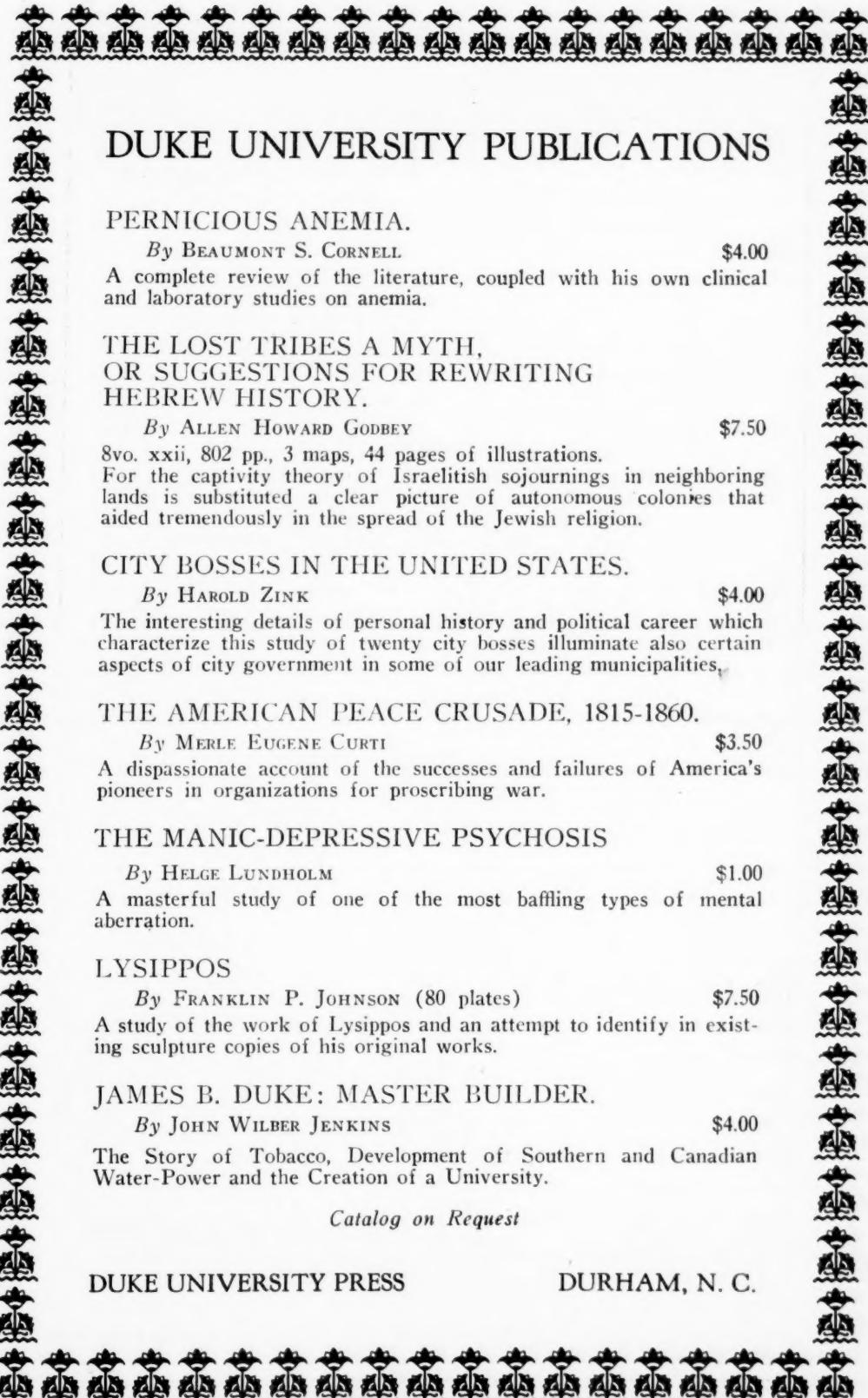
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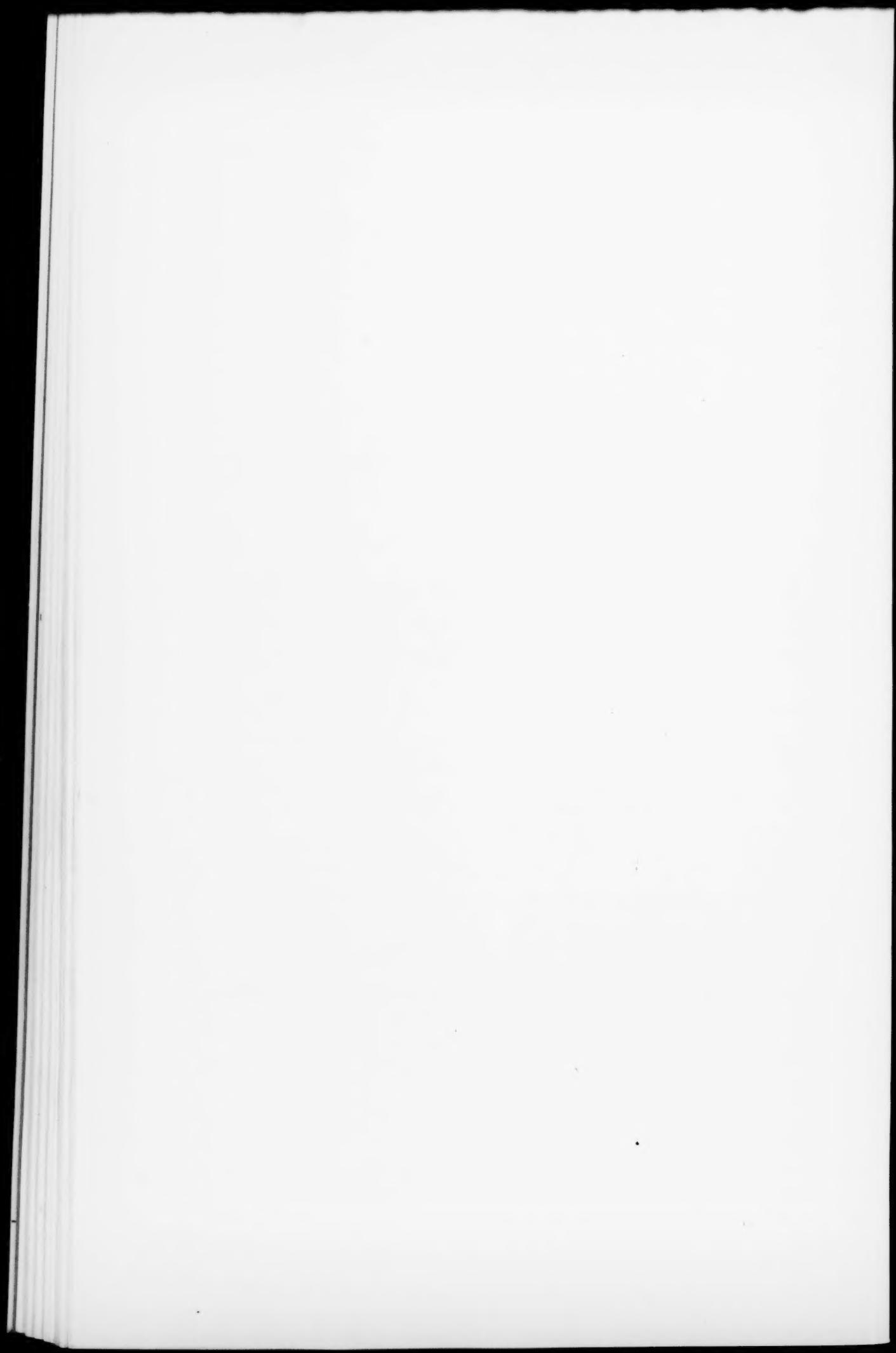
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